Specimen Collection and Analysis Considerations

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Outline

- **Biomonitoring**
- **Analyte vs biomarker**
  - *Analytical methods*
  - *Biomarker selection*
  - *Matrix considerations*
  - *Collection*
  - *Contamination (ubiquitous chemicals)*
  - *Variability*
- **Maternal vs child samples**

Assessing Early Life Exposures to Environmental Chemicals

- Questionnaire/Historical information
- Environmental monitoring (air, water, food, soil)
- Personal monitoring
- Biomonitoring

- Combine these approaches with calibrated and validated models

Biomonitoring

- *Assessment of internal dose by measuring the parent chemical (or its metabolite or reaction product) in human samples (e.g., blood, urine, milk)*
  - *Integrates all sources/routes of exposure*

Biomonitoring & Analytical Chemistry

- Complex & expensive instrumentation
- State-of-the-art laboratory facilities
- Highly trained personnel

Analytical Chemistry vs Biomonitoring

Analyte

- *Validated method*
  - *Analytical standard*
  - *Qualified personnel*
  - *QA/QC*
    - *Laboratory blanks*

Biomarker

- *Validated method*
  - *Analytical standard*
  - *Qualified personnel*
  - *QA/QC*
    - *Laboratory blanks*
- *Analyte metabolism & toxicokinetics*
- *Matrix considerations*
- *Sampling considerations*
  - *Field blanks*
  - *Timing/place of collection*

- *We do not measure exposure, but concentrations*

Analyte vs Exposure Biomarker

- *Additional information is needed to demonstrate the utility of measured analytes as exposure biomarkers*
- *Validated analytical methods, toxicokinetics knowledge, and adequate sampling*
  - *Selection of biomarker*
  - *Matrix considerations*
  - *External contamination*
    - *Biomonitoring integrates all sources/routes of exposure*

Selection of Exposure Biomarkers: DINP example

Unweighted number of participants (weighted percentage)		MCOP urinary concentrations		
		Detectable	*Non-detectable*	*Total*
MNP urinary concentrations	*Detectable*	*347(12.9%)*	*2 (0.02%)*	*349 (12.9%)*
	Non-detectable	*2100 (82.4%)*	*99 (4.7%)*	*2199 (87.1%)*
	Total	*2447 (95%)*	*101 (5%)*	

- **MNP & MCOP are both metabolites of DINP**
- **MNP: insensitive biomarker of background exposures to DINP (minor DINP metabolite)**
  - *82.4 % of those classified as exposed to DINP are misclassified as unexposed based on urinary concentrations of MNP only*
- **DINP exposure prevalence underestimated**

Calafat et al. EHP 2011, 119:50-55.

Matrix Choice

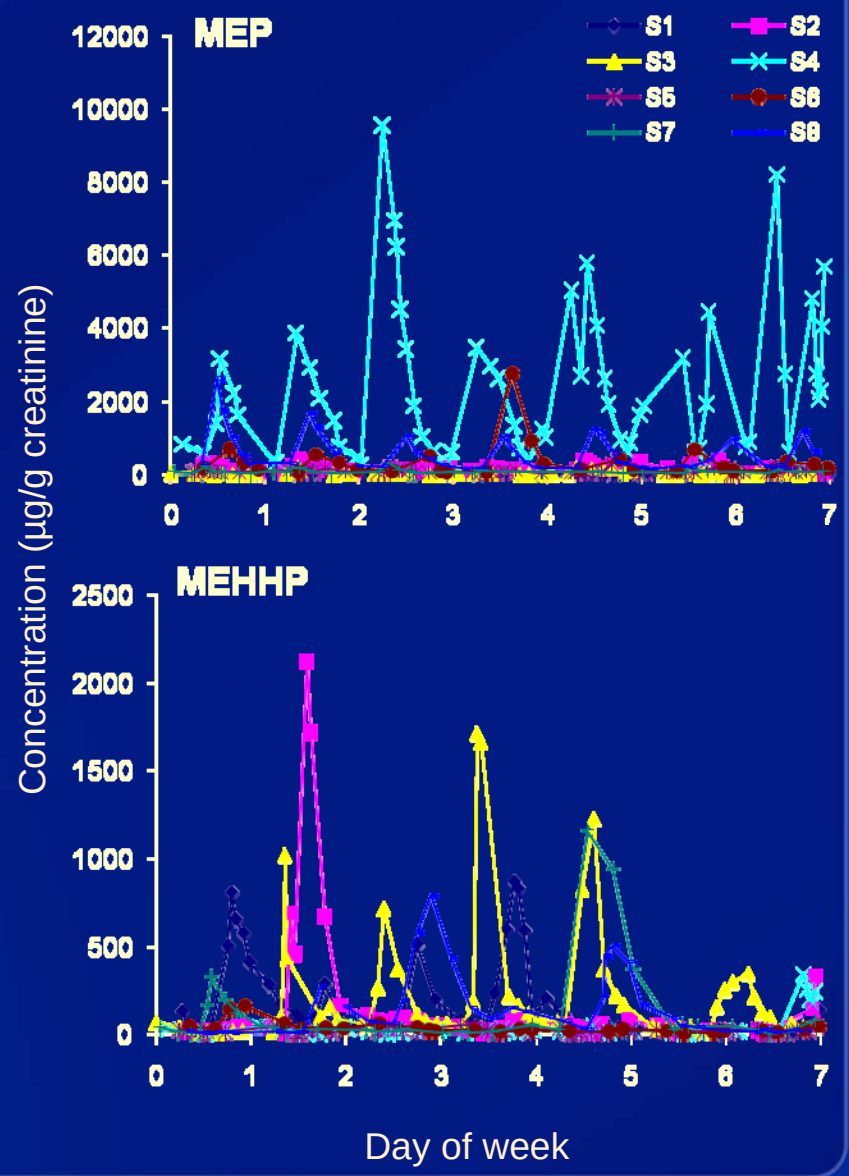
- *Matrix availability at a given life stage*
  - *Volume*
  - *Ability to collect matrix (e.g., fetus access)*
- *Persistence of biomarker*
  - *Persistent (blood)*
  - *Non-persistent (urine)*
    - *Variability in concentrations for non-persistent chemicals*
    - *Multiple samplings*
  - *Metabolism variation (e.g., age, genetics)*
- *Matrix composition may affect biomarker measures*
  - *Endogenous enzymes can hydrolyze phthalates (in the environment) to phthalate monoesters (biomarkers)*
    - *Acid to quench reaction?*
    - *Safety considerations*
  - *Measure only specific metabolites*
    - *Milk, serum, amniotic fluid, meconium, saliva*

What About Meconium?

- *Exploratory matrix*
  - *Distribution of target analytes*
- *Collection protocols*
  - *Avoid contamination of meconium with urine*
    - *Non-persistent chemicals*
  - *Set the time window of collection*
    - *Meconium vs other*
  - *Control meconium-diaper contact*
    - *Wet/dry weight*
- *Comparisons of biomarkers concentrations in meconium in epidemiologic studies should be conducted cautiously*
  - *Mainly semi-quantitative data*
  - *Limited use*

Variability in Biomarker Urinary Concentrations

- **8 adults (50% female)**
  - *All urine voids (N = 427) for 7 days*
  - *Variability in concentrations of phthalate metabolites*
    - *MEP (PCP use): <u>between-person</u> variability accounted for > 75% of total variance*
    - *MEHHP (diet): <u>within-person</u> variability contributed 69–83% of total variance*
    - *Spot samples intra-day variability:*
      - *MEHHP (51%)*
      - *MEP (21%)*



Preau et al. EHP 2010, 118(12):1748-54

Sampling Strategies

- *Does a single sample adequately characterize an individual's average exposure for a given time period?*
  - *24-h vs spot collections*
- *Varies depending upon the biomarker, exposure scenario and the population studied*
  - *For chronic exposures, probably*
  - *For episodic exposures, maybe, depending upon type (e.g., diet), frequency and magnitude of exposure*
    - *Time of collection and last urination for spot collections*
    - *Age-related variability*
- *Can we overcome variability?*
  - *Multiple urine collections per person*
    - *Cost (storage, analysis) & compliance considerations*
  - *"Pooling" several spot samples*

Collection & Storage Matter

- *Even with validated analytical methods, toxicokinetics knowledge & adequate sampling*
  - *Collection (how/when/where?)*
    - *Hospital /medical settings*
    - *Collection protocols (e.g., use of wipes, preservatives)*
  - *Integrity of specimen*
    - *Representativeness (absorbent collection materials)*
    - *Cross-matrix contamination (meconium/urine; AF/blood)*
    - *Stability of biomarker & matrix (e.g., conjugates, DBS)*
  - *Contamination*
    - *Measure trace levels of parent chemical (if ubiquitous & unidentified sources exist)*
    - *Add analytes post-collection – use of archived samples*
      - *Field blanks, blind replicates*

Calafat and Needham EHP 2009, 117:1481-5

Mothers' Prenatal & Postnatal Exposure

- *Maternal sample as surrogate for fetal/childhood exposure*
  - *Only if a relationship has been established*
- *Microenvironment changes rapidly over the first 5 years of life*
- *Are urine/serum levels good proxies for milk levels?*
  - *Serum/milk for some POPs*
    - *Lipophilicity (e.g., PCBs vs PFCs)*
    - *Serum levels not a good proxy measure for milk levels (PFCs)*
  - *Urine/milk for non-persistent pollutants*
    - *Matrix & chemicals properties*
    - *Urine levels not a good proxy measure for milk levels (phthalates)*

Facts to Remember

- **Biomonitoring is only one approach**
- **Maternal samples can be surrogates of fetal exposure**
  - *Known ability to transfer from the mother to the fetus?*
  - *Defined relationship between maternal & fetal chemical levels?*
  - *Multiple sampling for non-persistent chemicals*
- **Most likely, maternal exposures are not the best surrogates for childhood exposures, particularly as the child ages**
- **Cannot assume matrix is readily available from child**
- **Consider the importance of collection & storage procedures**

Final Thoughts

- **Must have validated analytical methods**
- **Selection of appropriate biomarkers**
  - *Must consider metabolism & matrix factors*
- **Collection & handling considerations**
  - *Stability (analyte & matrix)*
  - *Research potential contamination sources*
  - *Field blanks*
- **Going from concentration to exposure**
  - *Improve understanding of toxicokinetics of biomarker*
  - *Collection of additional data (e.g., time of last urination)*
  - *May need multiple collections*
- **Many analytes can be measured, but not all of them are good exposure biomarkers**

For more information please contact Centers for Disease Control and Prevention

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Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
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