Biomonitoring
Principles

Susan M. Pinney, PhD
Department of Environmental Health
Center for Environmental Genetics
University of Cincinnati College of Medicine
A. General information about biomarkers.
   • A **biomarker** is a chemical measure used to detect genetic, cellular or molecular alterations.

B. Specific information about environmental biomarkers.
   • An **environmental biomarker** is a measure of an environmental chemical or its metabolite in biomaedia. Represents internal exposure to the chemical.
Ideal Biomarker

1. Analytic method readily available
2. Reliably measured
3. Test conducted on easily obtainable biological materials (blood, urine)

For Disease Prediction:

1. Sensitive
2. Specific
3. Provide early detection so that intervention meaningfully impacts course of disease
4. Cost effective
Decisions, decisions.....
Choices vary by biomedia and what you want to measure.

• Collection
  – Sample type
  – Timing of collection

• Processing
  – Additives
  – Laboratory selection
  – Delayed processing

• Storage
  – Liquid nitrogen
  – Mechanical freezers

• Carefully selecting the biomarker and methods of your study minimizes mistakes and maximizes the information from your study!
Sample collection

- **Sex Hormones**
  - Similar whether collected in serum, EDTA plasma or heparinized plasma
  - Timing difficult in pre-menopausal women
    - Collect at early follicular and mid-luteal with postcard with date of menstrual period
    - Collect any day and provide specific dates of their cycle
    - Circadian rhythm for dehydroepiandrosterone (DHEAS), so time of day of blood collection

- **Ascorbic Acid and Carotenoids**
  - Can be measured in either serum or plasma and not affected by anticoagulant use.
  - Carotene and retinol have substantial seasonal variations. Control in design or in analysis
Sample collection, cont.

- **Inflammatory markers**
  - Most studies suggest that EDTA plasma is optimal
  - Little is known about the best time for collecting inflammatory biomarkers – might be more prevalent later in day.
  - Standardize time of data collection, such as for first morning sample.

- **Proteomics**
  - Serum samples may not be best for proteomic studies because
    - large number of serum-specific clot-related peptides produced.
    - can account for 40% of all peptide peaks in assays.
    - Anticoagulated plasma works better.
  - Fasting, time of day and medication use may all affect the measurement of proteomes.
Biologic half life and measured level
Short half-life versus long half-life

Figure source: Human Biomonitoring for Environmental Chemicals. www.nap.edu/catalog/11700.html.
Contribution of the half-life to the measured level

**Very short half-life:** Level in biomedia at time of sample represents concentration over the recent hours or days.

**Long half-life:** Level in biomedia at time of sample represents concentration over the recent months or years.

Figure source: ACGIH 1995. Topics in Biomonitoring: A compendium of Essays. Cincinnati, Ohio.
Sample Processing

• Processing should be:
  – Rigorous
  – Standardized

• Consider Feasibility – acceptable to participants, feasible logistics

• Alternate strategies
  – Plan for delayed processing – always happens in epidemiology studies.
Processing

• **Sex hormones**
  
  • Delayed processing up to 72 hours does not seem to affect concentrations

  • Sample should be aliquotted into airtight tubes to prevent degradation with long term freezing.

• **Ascorbic Acid and Carotenoids**
  
  • Carotenoids stable up to 1 week if blood kept chilled; at room temp can degrade.

  • Delay of >24 hours may degrade ascorbic acid levels even if samples are chilled.

  • May want to add an acid stabilizer such as metaphosphoric acid.

  • Oxidative damage with exposure to light, heat or oxygen (for either).
Processing, cont.

- **Inflammatory markers**
  - Samples kept refrigerated while processing
  - Can degrade or increase at room temp
  - Both interleukin-6 and tumor necrosis factor-α degrade after 4-6 hours at room temp.

- **Proteomics**
  - Need to be processed immediately if at room temp.
    - (Cool temps can activate platelets and release peptides into the sample)
  - May want to make the sample platelet poor by additional centrifuging or filtering.
  - Add protease inhibitor to reduce cleavage of proteins
    - Care in analyzing results: Molecular mass of protease inhibitors can overlap those of the proteome.
Freezer temp and long term storage

- **Liquid nitrogen freezers**
  - -130°C to -196°C (depending on whether samples are in liquid or vapor phase)

- **Mechanical freezers**
  - -20°C to -80°C
  - Study of 15 freezers, when temp was displayed as -81°C to -74°C, actual measurements -90°C to -43.5°C
  - Location in freezer

- Liquid nitrogen is preferable
Freezer temp and long term storage

- **Sex Hormones**
  - Store at -80°C
  - If stored at -20°C, sex hormone binding globulin may disassociate from estradiol and testosterone.
  - Increases measureable non-bound concentrations of these hormones.

- **Ascorbic Acid and Carotenoids**
  - Substantial decreases in carotenoid levels can occurred at -20°C for only 6 months; 97% decrease over 10 years.
  - Stable for up to 10 years at -80°C.
  - Long term storage of ascorbic acid requires an acid stabilizer.
  - Can assay after two or fewer freeze-thaw cycles
Freezer temp and long term storage

• **Inflammatory markers and proteomics**
  – Recommended storage at -80 to assure valid results.

• **Freeze thaw should be avoided:**
  – Inflammatory – may assay after up to six freeze-thaw cycles depending on the analyte
  – Proteome – also sensitive to freeze-thaw. Results in protein degradation.
Environmental Chemical

• An **environmental chemical** is a chemical compound or chemical element present in air, water, food, soil, dust, or other environmental media (e.g. consumer products).

• **Biomonitoring** is the assessment of human exposure to chemicals by measuring the chemicals or their metabolites in human specimens such as blood or urine.
Environmental Health Paradigm

Exposure

Biomarkers of Exposure

Environmental Measures of Exposure

Molecular Response (gene expression)

Biomarkers of Effect

Health Effects

Clinical Trials

“Environmental” Trials

Risk Communication

Health Policy

Prevention Through Exposure Reduction
Biomarkers

- **Biomarkers of exposure** indicate whether exposure to an agent has taken place, and include measurement of specific metabolites and/or adduct formed by reaction of the compound or its metabolites with macromolecules.

- **Biomarkers of susceptibility** can be used to identify specific individuals at greater risk than the general population as a result of genetic and other predisposition effects of exposure. These might include the activity of specific enzymes involved in activation or detoxification of a specific chemical or DNA repair capacity for specific types of DNA damage.

- **Biomarkers of effect** provide an indication of early events in development of toxicity, carcinogenesis or disease.
Different forms of monitoring and their relationship to exposure, dose, and effects.

<table>
<thead>
<tr>
<th>Metal Emissions</th>
<th>Exposure</th>
<th>Internal Dose Indicators</th>
<th>Biological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Sources</td>
<td>• Air</td>
<td>• Absorbed Dose</td>
<td>• Bioindicators of Effects</td>
</tr>
<tr>
<td>• Rates</td>
<td>• Water</td>
<td>• Body Burden</td>
<td>• Early Health Effects</td>
</tr>
<tr>
<td>• Patterns</td>
<td>• Soil</td>
<td>• Target Tissue Concentrations</td>
<td>• Overt Health Impairment</td>
</tr>
<tr>
<td>Source Characteristics and Emissions Monitoring</td>
<td>Environmental Monitoring</td>
<td>Biological Monitoring</td>
<td>Health Monitoring</td>
</tr>
</tbody>
</table>
Internal dose

- **Exposure assessment** - complete description of an environmental agent’s contact with and entry into the human body, including the sources in the environment, concentrations of the agent in the environment, pathways to the human body, and internal dose.

- **Internal (absorbed) dose.** The amount of the environmental agent absorbed by the human body, and therefore available to undergo metabolism, transport, storage, or elimination.

- **Biologically effective dose.** The portion of the dose of the environmental agent that reaches a certain human body tissue and the site or sites of toxic action within that tissue is the biologically effective dose.
Exposure Effect

- Water Perchlorate
- Blood Perchlorate uptake in thyroid
- Effective Dose
- Altered T3, T4, TSH
- Thyroid Histopathology
- Tumors

Susceptibility

Adapted from Schulte (1989)
In populations of workers or ill individuals levels of biomarker may be high and less sensitive assay is needed. Conversely, in community populations or well persons, more sensitive assay is needed. **KNOW THE capability and LOD of the lab before committing to a lab.**
More thoughts....

• **Some environmental chemicals pose challenge in study design.**

• **Chemical with short half life and source (and therefore concentration in serum or urine) varies by season, and daily within a season.**
  – Example: chemicals in sunscreen such as benzophenone-3 (BP-3) (also known as oxybenzone). Used mostly in summer, and daily use often varies with activities that day.
  – Best design to capture between-participant variability would be obtain multiple samples per person, and during season when exposure is greatest.

• **Urinary excretion of metabolites of parent compound.**
  – Assumption that what is excreted is proportional to internal exposure to parent compound.
  – Multiple metabolites.
    • Ratio of metabolites may be influenced by genetic variation.
    • Understand metabolic pathway.
    • Use sum of all or some of metabolites in data analyses.
Lessons learned…….

• Carefully research the biomarker you are considering.
• Hourly and daily variability
• Half-life and contributions of past time
• Optimal biomedia for measurement
• Use of anticoagulants or stabilizers at collection or processing
• Chemical stability at room temperature
• Temperature storage requirements
• Tolerable number of freeze-thaw cycles (volume of sample aliquots)
• Laboratory assay and limit of detection
• Choose wisely!
Acknowledgment

• This presentation was prepared by the Integrated Health Sciences Core of the University of Cincinnati Center for Environmental Genetics, supported by funding from the National Institute of Environmental Health Sciences (P30 ES 006096).