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BIOMARKERS & HUMAN BIOMONITORING

Children's Health and the Environment
WHO Training Package for the Health Sector
World Health Organization
www.who.int/ceh

October 2011

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Biomarkers and human biomonitoring

LEARNING OBJECTIVES

- ❖ To understand how biomarkers are used to assess environmental exposures
- ❖ To understand when and why biomarkers may be appropriate tools for specific situations
- ❖ To understand the advantage, limitations and challenges of biomonitoring
- ❖ To be able to give examples of how biomonitoring has been used effectively to improve environmental public health policy

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<<READ SLIDE>>

Biomarkers and human biomonitoring

DEFINITIONS

- ❖ Biomarker: a chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in the human body.
- ❖ Environmental monitoring: the measurement of a contaminant's concentration in a medium (e.g., air, soil, water, or food).
- ❖ Human biomonitoring: the direct measurement of people's exposure to toxic substances in the environment by measuring the substances or their metabolites in human specimens, such as blood or urine.

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For clarity here are the definitions of three major concepts.

<<READ SLIDE>>

While this talk concentrates on biomarkers and human biomonitoring, it is notable that it is also common to biomonitor other species – for example measuring methylmercury in fish is also biomonitoring as is measuring atrazine in frogs. Measuring contamination in other species can assist in developing both exposure risks for humans and information on toxicities.

Refs:

Definition for biomarker:

•WHO. Environmental Health Criteria 237. Principles for evaluating health risks in children associated with exposure to chemicals. WHO, 2006.

Definition for environmental monitoring:

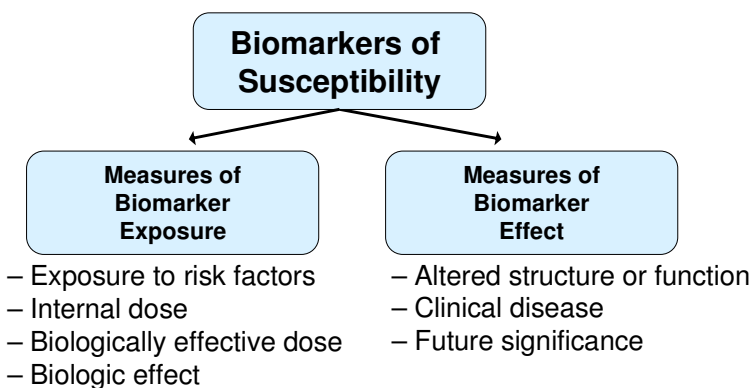
•Agency for Toxic Substances & Disease Registry. Case studies in environmental medicine. *Pediatric Environmental Health*, 2002, appendix F: 83. Available at www.atsdr.cdc.gov/csem/pediatric/appendixf.html#env – accessed March 2011

Definition for human biomonitoring:

•Centers for Disease Control and Prevention. National Biomonitoring Program. CDC. Available at www.cdc.gov/biomonitoring/ - accessed March 2011.

Biomarkers and human biomonitoring

Biomarker: a chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in the human body



Based on: Committee on Human Biomonitoring for Environmental Chemicals, 2006 4

The advantage of biomonitoring – the measurement of a chemical or its metabolite in the body – is that it represents an actual measure of integrated exposures via all routes of exposure which is not susceptible to assumptions or models.

Biomarkers are useful because they have the potential to measure the actual, integrated internal dose from all routes of exposure.

<<READ DEFINITION>>

There are several different categories of biomarkers, that measure exposure, effect and susceptibility (definitions below). Each is useful for answering different questions. In this presentation we are most concerned with biomarkers of exposure. In fact, there can overlap as the chemical makes its way down the metabolic cascade and into the body systems.

<<REVIEW CONTINUUM IN GRAPHIC>>

A biomarker of exposure: a chemical, its metabolite, or the product of an interaction between a chemical and some target molecule or cell that is measured in the human body (e.g. cotinine in blood or urine for second-hand tobacco smoke, benzene metabolites in urine for traffic-related pollution).

A biomarker of effect: A measurable biochemical, physiologic, behavioral, or other alteration in an organism that, depending on the magnitude, can be recognized as associated with an established or possible health impairment or disease (e.g. DNA adducts).

A biomarker of susceptibility: An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical substance. (e.g. G6PD deficiency)

Ref:

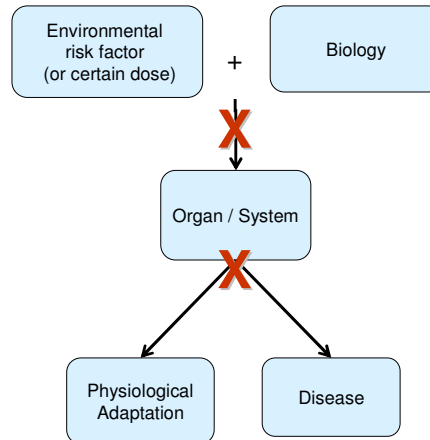
•Committee on Human Biomonitoring for Environmental Toxicants, National Research Council. Human biomonitoring for environmental chemicals. *National Academies Press*, 2006.

Image based on: Committee on Human Biomonitoring for Environmental Toxicants, National Research Council. *Human biomonitoring for environmental chemicals. National Academies Press, 2006.*

Biomarkers and human biomonitoring

ENVIRONMENTAL ILLNESS

- ❖ Defining the extent and impact of exposure is the key to understanding environmental illnesses
- ❖ No harm without exposure
- ❖ Exposure does not always mean harm



Based on: Agency for Toxic Substances & Disease Registry 2002

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<<READ SLIDE AS YOU CLICK THROUGH ANIMATION>>

Understanding environmental illness requires knowledge of the entire cascade of events from the release of an environmental contaminant through absorption, actions and damage within the body and the development of disease. Defining the extent and impact of exposure is a central element of understanding environmental disease.

Diagram: Environmental contamination and biological exposure will lead to absorption of an internal dose, followed by distribution, metabolism and excretion. Upon organ contact, either physiological adaptation will take place or a disease will develop.

<<CLICK FOR ANIMATION>>

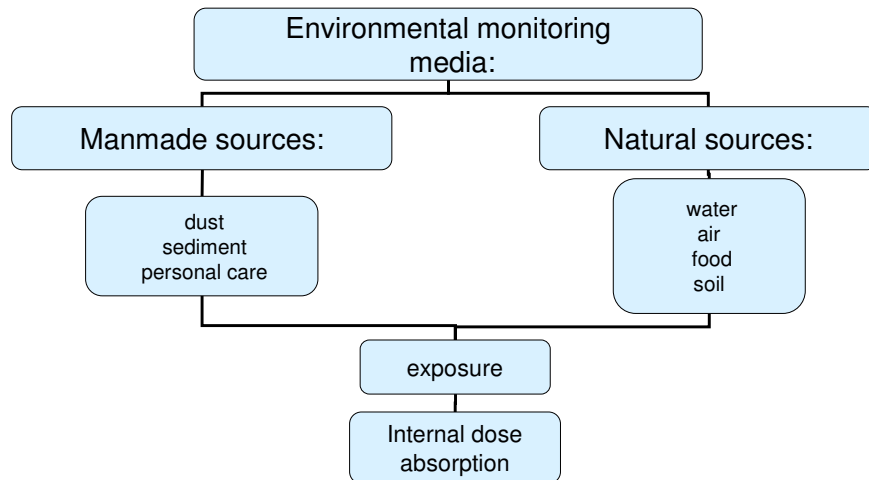
In simple terms, there can be no harm without exposure, but

<CLICK FOR ANIMATION>>

Exposure alone is not enough to prove or ensure harm.

Image based on: Agency for Toxic Substances & Disease Registry. Exposure-disease model. Case studies in environmental medicine. Pediatric Environmental Health, 2002, appendix A. Available at www.atsdr.cdc.gov/csem/pediatric/appendixa.html – accessed March 2011

Biomarkers and human biomonitoring



Based on: Needham, 2007

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<<REVIEW SLIDE BRIEFLY>>

The top portion is the realm of environmental monitoring – the measurement of pollutants and their breakdown products in various environmental media such as air, water, food, soil, and manmade objects.

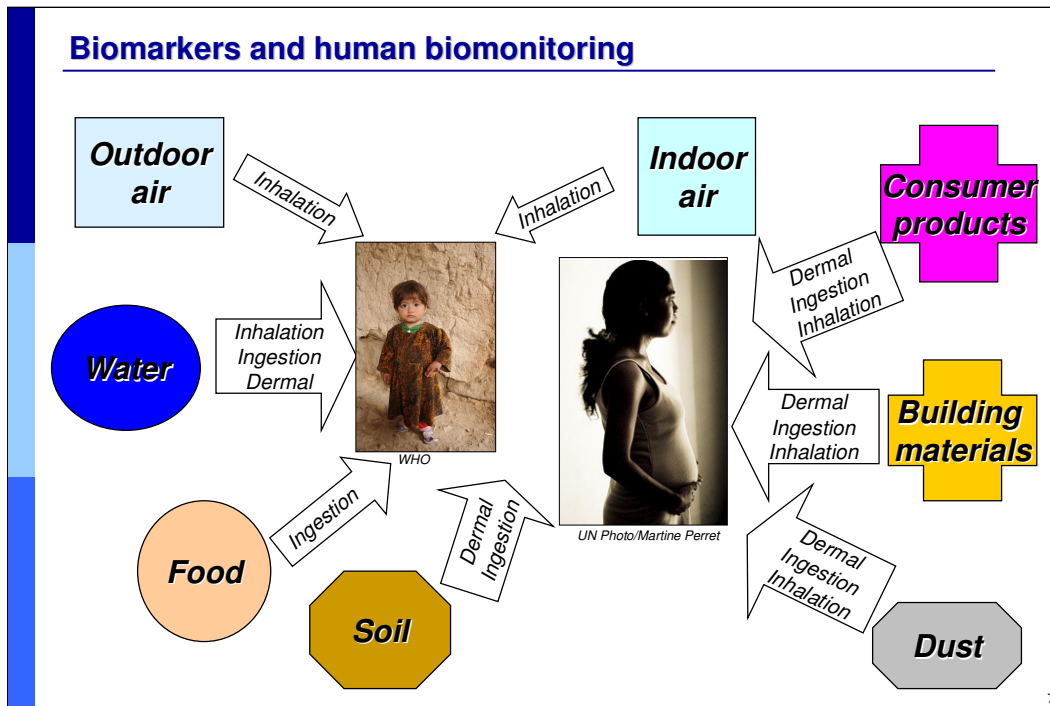
The lower portion is the realm of biomonitoring – the direct measurement of contaminants in the body.

In the middle, bioavailability modifies exposure routes or pathways – that is some contaminants are so tightly bound to their environmental medium that they are not available to be absorbed into the system by specific routes of exposure, which brings us finally to

EXPOSURE leading to absorption which is the key step which is often one of the most difficult to characterize.

This presentation will briefly describe biomarkers and environmental monitoring and exposure modeling, but is mostly concerned with the increasingly important tool of biomonitoring for understanding exposures.

Image based on: Needham LL et al. Uses and issues of biomonitoring. International Journal of Hygiene and Environmental Health, 2007, 210:229-238.



Environmental monitoring, also known as ambient monitoring, is the measurement of chemical substances in media (or matrices) like indoor and outdoor air, water, food, soil, dust, consumer products, building materials, etc. This is critical to understanding the sources of exposure, but is incomplete without information about

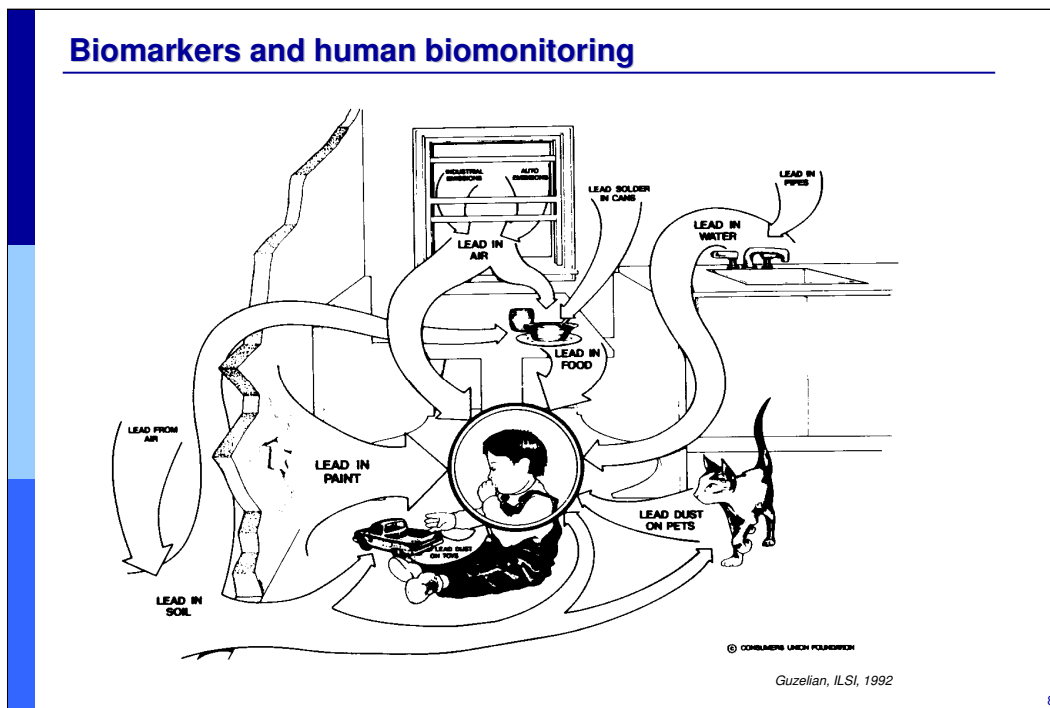
exposure pathways. In order to predict internal dose, complex exposure models are constructed which involve applying sets of standardized assumptions about activity levels, dietary choices, behavior, etc.

<<NOTE TO USER: The image of the pregnant woman is intentional. When considering exposures in children, it is always important to consider the “parenteral” exposure from mother to fetus. Mothers absorb environmental toxicants via the standard routes of inhalation, ingestion and dermal absorption, then the chemicals are transformed and many reach the fetus via the blood stream (some by diffusion across the placenta). These are qualitatively unique exposures which may pose serious risks to the developing fetus depending upon internal dose and timing.>>

Right image: United Nations. Martine Perret.

Left image: WHO. Afghanistan, 2010.

Biomarkers and human biomonitoring



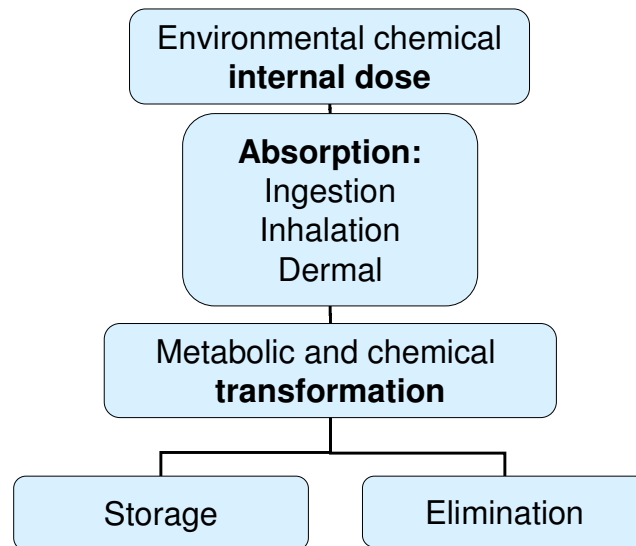
This picture shows the many different pathways by which a child can be exposed to environmental chemicals, in this case lead. Building exposure models based upon ambient monitoring requires making many assumptions about routes of exposure including quantitative importance of each route of exposure and rates of absorption into the body from each route. This is difficult for adults, but even more complex for children who are constantly growing and changing, have changing behavior patterns and live in changing living zones based upon size and activity. Exposure modeling for children requires utilization of a multi-lifestage approach and has multiple sources of potential error and uncertainty. Regulatory agencies and researchers are continuously revising and improving upon complex and extensive exposure models but there is always both uncertainty and error attached to exposure estimates generated with models.

Ref:

•WHO. Environmental Health Criteria 237. Principles for evaluating health risks in children associated with exposure to chemicals. WHO, 2006.

Image from Guzelian PS et al. Similarities and differences between children and adults. International Life Sciences Institute, 1992. Used with permission from International Life Sciences Institute.

Biomarkers and human biomonitoring



Modified from Needham, 2007

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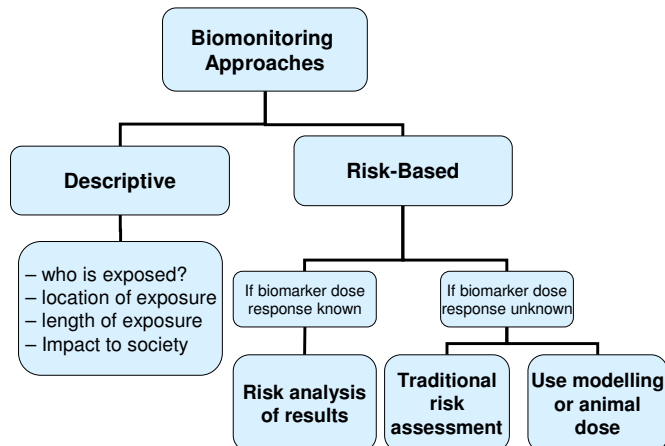
What is of physiological importance is the internal dose of the environmental chemical. That is the amount of drug absorbed via ingestion, inhalation, and dermal routes. Once inside the body, the chemical moves through the metabolic pathways of the body, is transformed and either stored or eliminated. (Note that *in utero* exposure is not included on this slide – clearly an important pathway for the fetus). There are various compartments that a xenobiotic (foreign chemical) may traverse. Implied are the various transformations that a chemical undergoes when subjected to the metabolic pathways of the body. In order to interpret a biomarker, it is necessary to know where in this process the measured biomarker comes from – that is whether it is a biomarker of exposure, susceptibility or effect as described on the previous slide.

Image based on: Needham LL et al. Uses and issues of biomonitoring. International Journal of Hygiene and Environmental Health, 2007, 210:229-238.

Biomarkers and human biomonitoring

WHY USE BIOMARKERS?

- ❖ Clinical uses
- ❖ Research uses
- ❖ Public health uses
- ❖ Policy uses



Based on: Committee on Human Biomonitoring for Environmental Chemicals, 2006 10

There are a variety of uses for biomarkers that are outlined in this schematic.

<<READ SLIDE>>

Biomarkers may be particularly useful when they provide linkage to important exposure, but must be measured in the correct matrix for exposure route/source of interest, and there must be analytic technology available, reliable, reproducible. Without proper study design, biomonitoring can be confusing at best and dangerous at worst.

Image based on: Committee on Human Biomonitoring for Environmental Toxicants, National Research Council. Human biomonitoring for environmental chemicals. National Academies Press, 2006.

Biomarkers and human biomonitoring

“The Matrix”

- ❖ Blood
- ❖ Urine
- ❖ Breast milk
- ❖ Expelled air
- ❖ Hair
- ❖ Nails
- ❖ Saliva
- ❖ Teeth
- ❖ Meconium
- ❖ Amniotic fluid
- ❖ Adipose tissue
- ❖ Other tissues and fluids



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<<READ SLIDE>>

Choice of the appropriate “matrix,” the body fluid or tissue to be tested, requires understanding the absorption and metabolism of the chemical of interest. Standardized collection, storage, processing and analytical protocols are critical for meaningful results. Blood, urine, breast milk and expelled air are used most commonly. Some matrices, such as hair and nails, are easily contaminated and difficult to collect in a standardized way. Therefore, results from lesser used matrices should be scrutinized carefully to ensure they are valid measures of the exposure under study, collected properly and analyzed by a certified lab. Finally, many biomarkers are not used or useful clinically at the individual patient level, but only for research purposes. One important reason for this is that for most environmental chemicals there are no “standard ranges” or “safe ranges” established for biomarkers. This is one of the many reasons that before ordering or interpreting a biomarker at the individual patient level, it is important to consult with experts in the field to be advised on the best way to assess environmental exposures or possible environmental illnesses in the individual patient. Increasingly there are environmental health specialists in many regions of the world who are excellent resources along with the global network of poison control centers.

Refs:

- European Human Biomonitoring. Frequently asked questions: what is human biomonitoring? Available at www.eu-humanbiomonitoring.org/sub/faq.htm – accessed March 2011

Human Biomonitoring is a scientific technique for assessing human exposures to environmental agents and their effects, based on sampling and analysis of an individual's tissues and fluids. While blood, urine, breast milk and expelled air are most commonly measured, hair, nails, fat, bone and other tissues may also be sampled. This technique takes advantage of the knowledge that environmental agents that have entered the human body leave markers reflecting this exposure. The marker may be the agent itself or a breakdown product, but it may also be some change in the body resulting from the interaction of the agent or its breakdown product(s) with the individual, such as alterations in the levels of certain enzymes or other proteins which may lead to modifications of normal body processes. (European Human Biomonitoring).

- WHO. World directory of poisons centres. Available at www.who.int/ipcs/poisons/centre/directory/en/ - accessed March 2011.

Chemicals or their metabolites can be measured in many different fluids or tissues.

Image: WHO. Drug Production, Bangkok.

Biomarkers and human biomonitoring

Chemical	Biomarker	Some interpretive options
Polybrominated diphenyls (PBDE)	PBDE in blood and breast milk	Identify exposed population, key information gaps, need for new toxicity and exposure data
Lead	Blood lead	Follow population exposures over time
Organo-phosphates	Parent compound, primary & secondary metabolites, blood and urine	Develop reference ranges, evaluate exposed subpopulations, evaluate public health interventions
Phthalates	Primary & secondary urinary metabolites	Develop reference ranges, identify and follow exposed subpopulations,
Dioxin	Dioxin in blood or lipid	Use of pharmacokinetic modeling to estimate body burden

Modified from Committee on Human Biomonitoring for Environmental Toxicants, 2006

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It is intended to illustrate the importance of matching the specific chemical biomarker and the matrix (or human fluid or tissue) in which it is analyzed with the specific questions that can be answered using the biomarker information. One very important function of biomonitoring is the development of “reference ranges” that describe general population exposures to contaminants. This provides context for more highly exposed groups and individuals, but with ubiquitous exposures may be misleading. As with lead poisoning in the mid 20th century, the “normal” level of population exposure did not produce acute illness and was thought to be “safe” but later found to be high enough to pose neurodevelopmental risks for children.

Because of the complexity of biomarker chemistry and environmental exposure and illness, it is possible to measure the wrong biomarker, obtain accurate but inappropriate information and draw false or misleading conclusions. Consulting with toxicology and environmental health experts to ensure that the biomarker is being appropriately chosen to answer the question of interest is critical. This list emphasizes biomonitoring in research and public health. The next slide gives examples of biomonitoring in clinical medicine.

This chart is simplified from *table 5.1 overview of major biomarker case examples used to illustrate interpretive options: chart*. At: Committee on Human Biomonitoring for Environmental Toxicants, National Research Council. *Human biomonitoring for environmental chemicals*. National Academies Press, 2006 - available at www.nap.edu/openbook.php?record_id=11700&page=138 – accessed March 2011.

Biomarkers and human biomonitoring

Chemical	Biomarker	Relative utility in clinical medicine
Lead	Blood Lead	Identify and manage lead poisoning in individual patients – very useful clinically,
Arsenic	Urinary Arsenic	Identify recent arsenic exposure – somewhat useful clinically
Organo-phosphates	Serum or red blood cell cholinesterase level	High intra- and inter-individual variability, overlap with toxic levels, results not available in a timely fashion, lab errors common, not useful clinically
Nitrate/nitrite	Methemoglobin	Nonspecific and expressed as % of total hemoglobin, must be interpreted within the context of full exposure history and physical exam – somewhat useful clinically
Benzene	Benzene in blood or expelled breath	Short half-life so only useful within a few hours of high exposure – not useful clinically outside of occupational setting

Modified from Committee on Human Biomonitoring for Environmental Toxicants, 2006

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This chart represents examples of biomonitoring that have variable utility in clinical medicine. There are many more examples. It is very important to consult with environmental health or toxicology specialists before interpreting biomonitoring data at the individual level. Many biomarkers that are useful for research purposes lack the precision necessary for clinical utility and are often highly sensitive to poor laboratory technique. If biomarkers are tested in non-specialty laboratories without knowledge of population reference ranges or toxic levels, or proper analytical and quality assurance techniques, they can be incorrectly interpreted and lead to medical management errors and ultimately patient harm.

For thorough treatment of clinical exposures to these chemicals, see ATSDR Clinical Environmental Case Studies at www.atsdr.cdc.gov/csem/conteduc.html

<<NOTE TO USER: There may be important exposure in your region which should be included in this section. Additional slides can easily be inserted here.>>

This chart is simplified from *table 5.1 overview of major biomarker case examples used to illustrate interpretive options: chart. At: Committee on Human Biomonitoring for Environmental Toxicants, National Research Council. Human biomonitoring for environmental chemicals. National Academies Press, 2006 - available at www.nap.edu/openbook.php?record_id=11700&page=138 – accessed March 2011.*

Biomarkers and human biomonitoring

LEVELS OF DETECTION (LOD) FOR CHEMICALS IN THE ENVIRONMENT AND THE BODY ARE NOW VERY LOW

- ❖ PPM (parts per million) mg/L or mg/kg
- ❖ PPB (parts per billion) $\mu\text{g/L}$ or $\mu\text{g/kg}$
- ❖ PPT (parts per trillion) ng/L or ng/kg
- ❖ PPQ (parts per quadrillion) pg/L or pg/kg

1 milligram (mg) = 1/1,000 gram = 0.001 gram
1 microgram (μg) = 1/1,000,000 gram = 0.000001 gram
1 nanogram (ng) = 1/1,000,000,000 gram = 0.000000001 gram
1 picogram (pg) = 1/1,000,000,000,000 gram = 0.000000000001 gram

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As analytical chemistry has advanced over the past decades, our ability to detect environmental chemicals in the human body has greatly increased. We are now able to measure very low levels of exposures routinely. This slide shows the increasingly smaller and smaller levels of detection than can now be possible for various chemicals. One critical area of evolving science is the determination of what a “significant exposure” is (that is carrying risk of disease/illness/damage) versus what is a “measurable exposure”. This information for the vast majority of environmental chemicals is unknown and particularly complex for childhood exposures which can appear minimal but occur at critical points in development and cause damage that manifests in adulthood.

<<NOTE TO USER: Examples of what these low levels of detection represent quantitatively are listed below in the analogies:>>

PPM (parts per million) mg/L or mg/kg

(1 cup of water in a swimming pool)

PPB (parts per billion) $\mu\text{g/L}$ or $\mu\text{g/kg}$

(1 drop of water in a swimming pool)

PPT (parts per trillion) ng/L or ng/kg

(1 second in nearly 32,000 years)

PPQ (parts per quadrillion) pg/L or pg/kg

(1 second in nearly 32 million years)

Refs: Analogies come from:

•United States Environmental Protection Agency

•University of Minnesota. Measurement units and concentration analogies. Available at www.seagrant.umn.edu/water/report/helpfulinformation/concentrations.pdf - accessed March 2011.

Biomarkers and human biomonitoring

Examples of order of magnitudes for levels of detection for some biomarkers

Marker	Matrix	Units
Polycyclic aromatic hydrocarbon	Urine	ng/L
Cotinine	Serum	ng/mL
Benzene	Blood	ng/mL
Organophosphate metabolites	Urine	µg/L
Arsenic	Urine	µg/L
Bisphenol A	Urine	µg/L
Lead	Blood	µg/dL
Polybrominated diphenyl ethers	Serum	ng/g lipid
Dioxin	Serum	pg/g lipid

Chart abstracted from www.cdc.gov/exposurereport/data_tables/appendix_d.html 15

<<NOTE TO USER: In the U.S., cost varies widely from a few U.S. dollars for lead and mercury to thousands of U.S. dollars for dioxins and PCBs. Please identify cost data pertinent to your population and region.>>

Human biomarkers also vary greatly in price. This chart displays some common biomarkers and their levels of detection in body fluids. Costs will vary from laboratory to laboratory and country to country, but measuring many environmental chemicals in the body can be quite expensive.

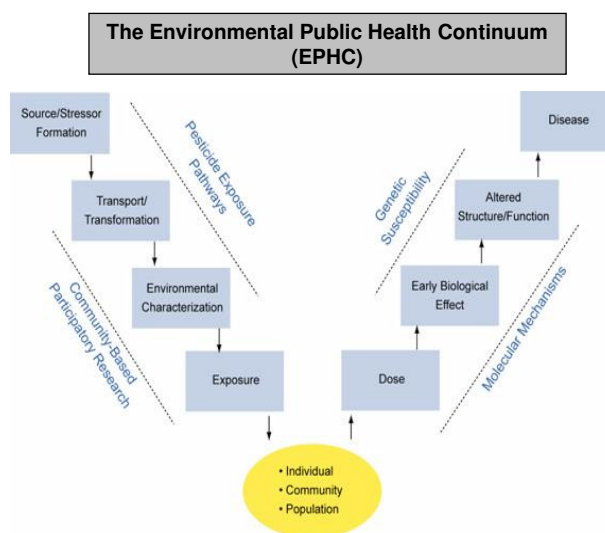
LOD and matrix columns are abstracted from: Centers for Disease Control and Prevention. National report on human exposure to environmental chemicals. Fourth report. CDC, Appendix D, limit of detection table, available at www.cdc.gov/exposurereport/data_tables/ - accessed March 2011.

LOD: Levels of detection

Biomarkers and human biomonitoring

Biomarkers are most useful when both “up stream” and “down stream” knowledge is complete

- ❖ Primary sources of environmental contaminant understood
- ❖ Pathways/routes of exposure understood
- ❖ Human exposure is related to animal toxicology studies
- ❖ Exposure-dose relationship understood
- ❖ Timing and duration of exposure known



US Environmental Protection Agency

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Just as exposure modeling has uncertainties, so do biomarkers. They are most useful when the entire causal chain from source to disease is well described.

<<READ SLIDE AND REVIEW GRAPHIC>>

The source of the environmental contaminant is important to understand, along with the means of transport and exposure-dose relationship. This aids in understanding the early biologic effect that alters the structure and function and ultimately leads to disease.

Image: US Environmental Protection Agency, University of Washington Center for Child Environmental Health Risks Research, available at www.epa.gov/ncer/childrenscenters/washington.html – accessed March 2011

Copyright notice: works produced by the U.S. Government are in the public domain.

Biomarkers and human biomonitoring

Advantages of biomarkers

- ❖ Confirms absorption into the human body
- ❖ Measures integrated exposure
- ❖ Very low level exposures detectable
- ❖ Helps to test and validate exposure models
- ❖ Helps to follow exposure trends
- ❖ Helps to evaluate public health interventions

Limitations of biomarkers

- ❖ Does not define sources, pathways or duration of exposure
- ❖ Cannot define toxic dose
- ❖ Susceptible to inferior or unscrupulous analytical laboratories
- ❖ Lack of meaningful reference levels
- ❖ Lack of toxicological and epidemiological information about the vast majority of environmental chemicals

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As with any technology, there are advantages and disadvantages of biomarkers, as summarized in the slide.

Advantages of biomarkers

- Confirms absorption into human body. Measures integrated exposure from all routes and all sources – not dependent upon models or assumptions.
- Very low level exposures detectable because analytical techniques have become exquisitely sensitive over the past several decades.
- Help to test and validate exposure models when the results of modeling predictions are compared to internal doses actually measured in exposed individuals.
- Help to follow exposure trends when individuals or representative samples of groups are followed with serial biomarker testing over time.
- Help to evaluate public health interventions, that is if chemicals are withdrawn, restricted or products are reformulated, follow up biomonitoring can determine if exposure has actually decreased.

Limitations of biomarkers

- Does not define sources or pathways of exposure – because it is a snap-shot and an integrated measure, it tells us nothing about where the chemical came from or how it got into the body.
- Cannot define toxic dose – unless toxicology and epidemiology studies have defined toxicity and the dose response curve, the simple presence of a chemical in an individual may be difficult to interpret.
- Susceptible to inferior or unscrupulous analytical laboratories – because by definition environmental chemicals and pollutants tend to be ubiquitous, and many chemicals of interest are used in every day products including laboratory equipment, it is possible that samples will be contaminated during collection and processing. Proper procedures are imperative to ensure interpretable results. Unqualified commercial laboratories may not only have poor techniques, they may also test for the wrong things in the wrong kinds of samples.
- Lack of meaningful reference levels – for many pollutants population reference levels are not known. If the exposure is ubiquitous, average population level exposure may be above the toxic limit, but be considered “normal” (as in the case of blood lead levels in the middle of the 20th century).
- Lack of toxicological and epidemiological information about the vast majority of environmental chemicals – of the 80,000-90,000 manmade chemicals in the world today, less than half have ever been tested for toxicity of even the most simple kind. There is a tremendous information gap which makes it often impossible to know if exposures measured by biomarkers are “dangerous” or “safe”.

Biomarkers and human biomonitoring

METHODOLOGICAL ISSUES

- ❖ Analytical technique
- ❖ Environmental contaminants and controls
- ❖ Laboratory contamination and quality assurance
- ❖ Correct choice of biomarker for study design and question
- ❖ Rationale for selecting environmental chemicals of interest
- ❖ Coordination with related research – epidemiology, toxicology, pharmacokinetic modeling, exposure assessment



WHO

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As discussed on the previous slides, the choice and handling of the specific specimen type and biomarker to be measured is highly complex. Some of the important considerations are listed here – all of these issues must be thoroughly addressed for a project to be maximally effective and useful.

<<READ SLIDE>>

Ref:

•Calafat AM, Needham LL. What additional factors beyond state-of-the-art analytical methods are needed for optimal generation and interpretation of biomonitoring data?. *Environmental Health Perspectives*, 2009, 117(10):1481.

interpretation of data: do collection protocols matter?

Biomonitoring (i.e., measurement of environmental chemicals, their metabolites, or specific reaction products in human biological specimens) to assess internal exposure (i.e., body burden) has increased considerably in the last two decades (Needham et al. 2007). Biological matrices are complex; some may be difficult to obtain and available only in small amounts. Moreover, environmental chemicals are normally present in the biological matrix at trace levels. Therefore, highly sensitive, specific, and selective multianalyte methods for the extraction, separation, and quantification of these chemicals must be developed (Needham et al. 2005). Undoubtedly, the adequacy of biomonitoring data depends strongly on reliable analytical measurements (Angerer et al. 2007). Even when the best techniques are used, they guarantee accurate and precise measures of the biomarkers levels only in any given specimen. However, if the integrity of the specimen was compromised before its analysis, the analytical measures, although valid, could lead to erroneous interpretations. Sampling, storage, and processing conditions have long been appreciated as potential sources of contamination in trace analyses for metals and volatile organic compounds (Ashley et al. 1992; Bolann et al. 2007; Pineau et al. 1993). Unfortunately, adequacy of sampling and processing methods, albeit critical for the evaluation of all biomonitoring data, has not received as much attention as the analytical techniques, especially for semivolatile organic chemicals.

Biomarkers and human biomonitoring

RISK COMMUNICATION ISSUES

Who gets the results and why?

- Exposures need context
 - Source and route
 - Bioavailability
 - Toxicity



WHO

At risk communities may have unrealistic expectations

- Lag between research and intervention

Why biomarkers are not always useful

- Incomplete knowledge of toxicity
- Inappropriate clinical use of research tools

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<<NOTE TO USER: Please replace with regionally appropriate photo.>>

For the vast majority of environmental chemicals found in the human body, little or no toxicology/epidemiology is available with which to interpret the meaning, which presents tremendous ethical and communications challenges! Non-scientists often do not understand that our ability to measure a chemical in the body at very low levels very often outstrips our knowledge about what that actually means. Because of this large knowledge gap, excellent risk communication becomes critical. Similarly, being able to measure even a known toxicant in the body, usually does not tell us where it came from or the specific route of exposure. (Rarely a specific isotope or chemical signature will identify a specific source but this is the exception rather than the usual condition). The context of the exposure must be fully defined before risk can be assessed. While that context is being developed there is a tremendous need to communicate frankly with screened populations.

When biomonitoring is being conducted in a “high risk” population, there may be expectations that the results of the study will generate definitive action that will improve that specific communities public health risk. It is critical to work with such communities to develop mutually acceptable understanding of what the likely outcomes will be and to facilitate communication with policy makers and local public health officials as much as possible.

Ref:

•Sly PD et al. Ethical issues in measuring biomarkers in children’s environmental health. *Environmental Health Perspectives*. 2009, 117(8):1187.

Community expectations

Research using biomarkers is carried out to learn about what a particular biomarker can tell the researchers about environmental exposures, susceptibility, or risks for adverse health outcomes from those exposures. Thus, such research is generally undertaken in exposed communities, which may raise expectations within the community that the study will per se improve the situation in the community (Eskenazi et al. 2005). However, the reality is that, in many cases, the risks are poorly understood and the primary purpose of conducting the study is to clarify the associations between exposures and health consequences and the role of biomarkers in understanding these associations. As pointed out by Eskenazi et al. (2005), understanding the community expectations and developing a communication strategy before starting the research is an important part of biomarker research. These issues are highlighted in two case reports outlining studies in children exposed to pesticides [Children’s Environmental Exposure Research Study (CHEERS)] or lead (Kennedy Krieger), which are included in the Supplemental Material (doi:10.1289/ehp.0800480.S1). These cases provide valuable lessons for conducting environmental health research in a community. (Sly PD, et al.)

Image: WHO. Nepal.

Biomarkers and human biomonitoring

ETHICAL ISSUES

- ❖ Informed consent
- ❖ Ability to inform dangerously exposed/at risk individuals
- ❖ Biobanking of genetic materials
- ❖ Ethical standards differ between researchers and community
 - Individual value versus community value
 - Industrialized versus developing nations
- ❖ Conflicts of interest



Centers for Disease Control and Prevention

WHO

WHO

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A variety of ethical issues arise when dealing with biological sampling, particularly when children are being sampled. Some of these issues are similar to general issues of research involving children such as gaining informed consent through surrogates (parents or caregivers). Others are more unique to biomonitoring. For example, most research protocols now demand that samples be anonymized to protect the privacy of research subjects, but this can be a problem if dangerous exposure levels are found. Biomonitoring often involves getting genetic material and banking it long term - so questions arise as to how this should be handled when samples are from minors. In addition, many biomonitoring studies are conducted by researchers from outside of the study population's country giving rise to conflicting basic standards and values. These issues are well explored in the paper by Sly et al referenced below.

Ref:

•Sly PD et al. Ethical issues in measuring biomarkers in children's environmental health. *Environmental Health Perspectives*. 2009, 117(8):1187.

The study of biomarkers in children raises a number of special ethical considerations related to the collection and storage of specimens, consent, and how to convey information about risk, especially where the level of scientific knowledge is inadequate to quantify that risk (Eskenazi et al. 2005). Many of these issues are similar to those involving adults (Caulfield et al. 2007; Evans and Meslin 2006; Helft et al. 2007; Malkin 2004), but other issues may be unique to children (Neidich et al. 2008). In this commentary we concentrate on issues specifically related to children rather than general issues.

Pediatric biomarker research is especially worthy of special ethical scrutiny, because it invokes issues arising in pediatric research generally coupled with those of environmental health—biobanking and genetics research more specifically. Moreover, when these types of studies are carried out in economically developing countries, further ethical issues emerge. (Sly PD, et al.)

Left image: Centers for Disease Control and Prevention. Children with dump truck, available at www.cdc.gov/exposurereport/index.html – accessed March 2011

Centre image: WHO. Afghanistan.

Right image: WHO. Mother with Baby.

Biomarkers and human biomonitoring

MANY NATIONAL & REGIONAL BIOMONITORING PROGRAMS EXIST

- ❖ In the U.S., decades of biomonitoring
- ❖ Increasing number of environmental chemicals monitored
- ❖ Increasing number of programs and agencies involved



WHO

21

<<NOTE TO USER: Insert here information about your local or regional biomonitoring program.>>

Biomonitoring programs are developing rapidly around the globe. The longest running programs are in industrialized nations.

Image: WHO. Mexico.

Biomarkers and human biomonitoring

EXAMPLES OF BIOMONITORING PROGRAMS

In the U.S.

- ❖ **HEI:** Human Exposure Initiative
- ❖ **HHANES:** Hispanic Health & Nutrition Examination Survey
- ❖ **NHATS:** National Human Adipose Tissue Survey
- ❖ **NHANES:** National Health & Nutrition Examination Survey
- ❖ **NHEXAS:** National Human Exposure Assessment Survey

In Europe & Canada:

- ❖ **Canada:** Health Canada's biomonitoring initiatives
- ❖ **European Union:** European Human Biomonitoring
- ❖ **Germany:** Human Biomonitoring Commission
- ❖ **Sweden:** Swedish Environmental Protection Agency on Environmental Pollutants

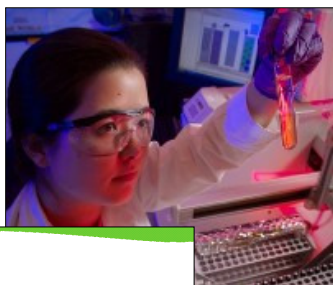
22

This slide shows biomonitoring programs in the U.S., Canada and Europe:

- Canada: www.chemicalsubstanceschimiques.gc.ca/plan/surveil/index-eng.php – accessed March 2011
- European Union: www.eu-humanbiomonitoring.org/index.htm – accessed March 2011
- Germany: www.umweltbundesamt.de/gesundheit-e/monitor/index.htm and www.uba.de – accessed March 2011
- Sweden: www.naturvardsverket.se/en/In-English/Start/State-of-the-environment/Environmental-pollutants/ – accessed March 2011

<<NOTE TO USER: Insert here information about your local or regional biomonitoring program>>

Biomarkers and human biomonitoring

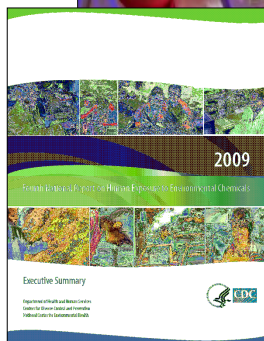


❖ NHANES

- Began in 1971, large, nationally representative sample
 - Interviews and physical exams
 - Subset gets biomonitoring
- Excellent for identifying population level exposures and trends

❖ National Biomonitoring Program

- National Exposure Report
 - Biannual, representative sample
 - Separated by age, sex, race/ethnicity



US CDC

US CDC

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<<NOTE TO USER: Here is an example slide presenting some programs in the U.S. Users should insert programs from their own country and region in this section of the presentation.>>

Refs:

- CDC. National report on human exposure to environmental chemicals. CDC, available at www.cdc.gov/exposurereport/index.html – accessed March 2011.
- NHANES: CDC. National Health and Nutrition Examination Survey. CDC, available at www.cdc.gov/nchs/nhanes.htm - accessed March 2011.

Here are a few details on 2 of the major biomonitoring programs in the U.S.

The National Health and Nutrition Examination Survey is a population-based survey designed to collect information on the health and nutrition of the U.S. household population. You may have come across data gathered through NHANES during your professional education. NHANES data have been used to influence policy and improve the health of the U.S. population in many ways including: getting lead removed from gasoline; creating and updating the pediatric growth charts; and establishing national baseline estimates for cholesterol, blood pressure, and Hepatitis C in the U.S.

There are two parts to this survey: the home interview and the health examination. We obtain written informed consent from each participant for both the in-home interview and the health exam. (NHANES).

The Fourth National Report on Human Exposure to Environmental Chemicals is the most comprehensive assessment to date of the exposure of the U.S. population to chemicals in our environment. CDC has measured 212 chemicals in people's blood or urine—75 of which have never before been measured in the U.S. population. The new chemicals include acrylamide, arsenic, environmental phenols, including bisphenol A and triclosan, and perchlorate. The blood and urine samples were collected from participants in CDC's National Health and Nutrition Examination Survey (NHANES), which is an ongoing survey that samples the U.S. population every two years. Each two year sample consists of about 2,400 persons. The Fourth Report includes findings from national samples for 1999–2000, 2001–2002, and 2003–2004. The data are analyzed separately by age, sex and race/ethnicity groups. (National Report on Human Exposure to Environmental Chemicals (the National Human Exposure Report))

Top image: CDC. National report on human exposure to environmental chemicals, available at www.cdc.gov/exposurereport/index.html – accessed March 2011.

Bottom image: CDC. Fourth National Report on Human Exposure to Environmental Chemicals, 2009, available at www.cdc.gov/exposurereport/pdf/FourthReport.pdf – accessed March 2011.

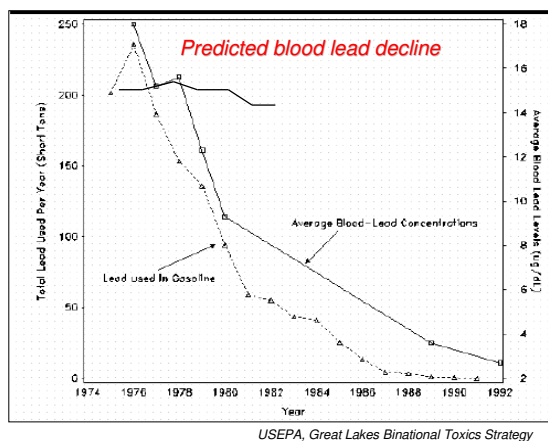
CASE STUDIES

<<NOTE TO USER: Look for case studies that are pertinent to your population and region. The following case studies are offered as examples of successful uses of biomonitoring but there are many others which may be more pertinent to your population. >>

Biomarkers and human biomonitoring

CASE STUDY: LEADED GASOLINE & BLOOD LEAD LEVELS - U.S.

- ❖ Lead removed from gasoline
 - Technical standard
 - To protect catalytic converters in automobiles
 - Health standard
- ❖ Blood lead levels in children fell in parallel with lead in air
 - Much more than models predicted
- ❖ New understanding of important sources of lead exposure in children



25

One of the most frequently quoted examples of public health success is the fall of population blood lead levels in the U.S. in parallel with phasing lead out of gasoline. Lead was removed from gasoline for the dual purpose of reducing lead exposure and protecting the catalytic converters required in cars to meet emission standards for certain criteria air pollutants (other than lead). What is remarkable about this story is that at the time the phase out began, few experts believed that air-borne lead contributed significantly to the lead burden in the community. When lead levels fell much more than predicted, it was appreciated that lead from gasoline was indeed a major contributor to the lead problem in the United States. Thus, biomonitoring was useful not only to document reduced population exposure, but also to inform revised environmental exposure modeling which more correctly identified air and contaminated dust as important sources of lead at the population level.

Ref:

An interesting perspective on the political process behind deleading gasoline in the USA:

•Bridbord K, Hanson D. A personal perspective on the initial federal health-based regulation to remove lead from gasoline. *Environmental Health Perspectives*, 2009, 117(8):1195-1201.

Image: USEPA, Great Lakes Binational Toxics Strategy at:

www.epa.gov/glnpo/bnsdocs/98summ/alead/fourthdraft.htm – accessed March 2001.

Modified approximating graphic on CDC flash presentation: www.cdc.gov/biomonitoring/ - accessed March 2011.

Biomarkers and human biomonitoring

CASE STUDY: LEAD & GASOLINE

Multiple countries around the world have removed lead in gasoline with similar reductions population exposure



www.free-stockphotos.com/images/pumping-gas.jpg

26

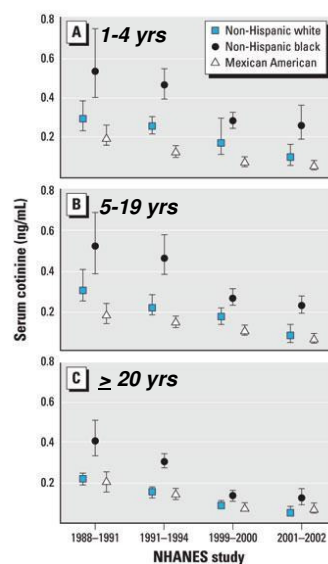
This experience has been repeated in most of the industrialized countries of the world and is beginning in developing nations as well.

Image: Free-StockPhotos.com. Pumping gas, at www.free-stockphotos.com/images/pumping-gas.jpg – accessed March 2011.

Biomarkers and human biomonitoring

CASE STUDY: COTININE & SECOND-HAND TOBACCO SMOKE

- ❖ Cotinine found in children at higher levels than adults
 - Health harms documented
- ❖ Smoking bans in public places proliferate
 - Population exposures fall 70%
- ❖ Biomonitoring identifies populations at differential risk
 - Children, non-Hispanic blacks
 - Improved opportunities for targeted intervention
- ❖ Bans on smoking in public effective



Pirkle, Environ Health Perspectives, 2006

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Pirkle, using NHANES data, analyzed the serum cotinine levels in non-smokers and found a 70% decrease over a 14 year period. This case illustrates several tenants of children's environmental health: children have higher exposures than adults; children are dependent upon adults to provide a healthy environmental where they live, play and learn; public health interventions (education, smoking cessation, bans on smoking in public places) can improve safety and reduce exposure and risk.

Refs:

- Centers for Disease Control and Prevention. National report on human exposure to environmental chemicals. CDC, available at www.cdc.gov/exposurereport/data_tables/Cotinine_ChemicalInformation.html - accessed March 2011.
- European Human Biomonitoring. European Environment and Health Action Plan, available at www.eu-humanbiomonitoring.org/sub/back/ehap.htm - accessed March 2011.

Cotinine is a metabolite of nicotine that can be measured in blood and urine. Cotinine levels are used to track exposure to environmental tobacco smoke (ETS) among non-smokers and to estimate exposure to ETS in every day-life situations on an individual basis and also on children. Higher cotinine levels indicate more exposure to ETS, which has been identified as a human carcinogen. (European Environment and Health Action Plan).

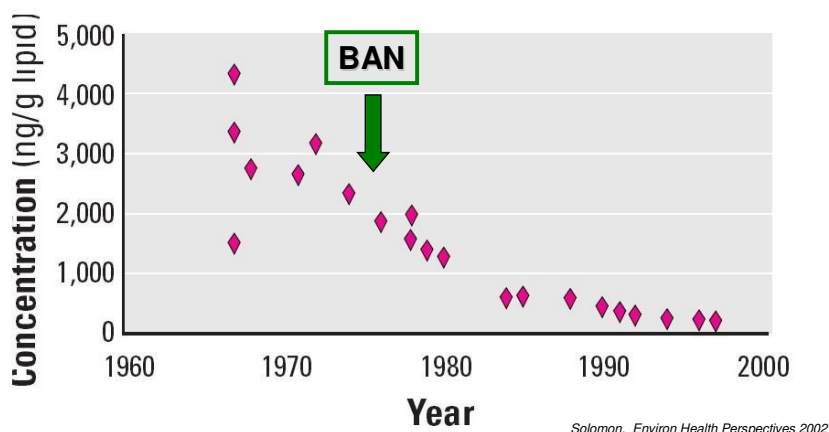
Serum cotinine levels reflect recent exposure to nicotine in tobacco smoke. Nonsmoking is usually defined as a serum cotinine level of less than or equal to 10 ng/mL (Pirkle et al., 1996). The serum cotinine levels seen in the NHANES 2003–2004 appear approximately similar to levels seen in the previous survey period (NHANES 2001–2002) for the total population estimates. Serum cotinine has been measured in many studies of nonsmoking populations, with levels showing similar or slightly higher results (depending on the degree of ETS exposure) than those reported in the previous NHANES (CDC 2005; NCI, 1999). Over the previous decade, levels of exposure to ETS appeared to decrease since geometric mean cotinine serum concentrations in nonsmokers had fallen by approximately 70% and the rate of detectable cotinine in nonsmokers fell from 88% to 43% when NHANES 1988–1991 was compared to NHANES 1999–2002, (CDC, 2005; Pirkle et al., 2006). The overall decline in population estimates of serum cotinine likely reflects decreased ETS exposure among nonsmokers in locations with smoke-free laws (Pickett et al., 2006; Soliman et al., 2004). During each previous NHANES survey, the adjusted geometric mean serum cotinine was higher in children (aged 4–11 years) than in adults among both non-Hispanic blacks and non-Hispanic whites (Pirkle et al., 2006). Non-Hispanic blacks had higher serum cotinine concentrations compared with either non-Hispanic whites or Mexican-Americans. Higher levels of cotinine have previously been reported for non-Hispanic black smokers (Caraballo et al., 1998). Differences in cotinine concentrations among race/ethnicity and age groups may be influenced by pharmacokinetic differences as well as by ETS exposure (Benowitz et al., 1999; Hukkanen et al., 2005; Wilson et al., 2005). Biomonitoring studies of serum cotinine will help physicians and public health officials determine whether people have been exposed to higher levels of ETS than are found in the general population. Biomonitoring data can also help scientists plan and conduct research about exposure to ETS and about its health effects. (Centers for Disease Control and Prevention).

Image: Pirkle LP et al. Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. Environmental Health Perspectives. 2006, 114:853-858. Copyright notice: This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original DOI.

Biomarkers and human biomonitoring

CASE STUDY: DDT IN BREAST MILK - SWEDEN

Bans can be effective in reducing human exposure



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DDT was severely restricted in Sweden in 1970 and completely banned in 1975. Biomonitoring of breast milk proves the efficacy of bans on reducing human exposure.

Ref:

•Solomon GM, Weiss PM. Chemical Contaminants in Breast Milk: time trends and regional variability. *Environmental Health Perspectives*, 2002, 110(6):339-347.

DDT. DDT (dichlorodiphenyltrichloroethane) is a commercial organochlorine insecticide that has been widely used on agricultural crops as well as for vector control. DDT and its by-products can persist in soil and sediments for more than 15 years and are known to bioaccumulate in animal tissues. As of 1995, DDT had been banned for all uses in 49 countries and restricted to vector control in 23.

The half-life of DDT in humans is approximately 4 years. DDT's major metabolite, dichlorodiphenylchloroethane (DDE), has a half-life of approximately 6 years. The relative proportion of DDT and DDE detected in human tissues can be an indication of the length of time since exposure. In areas where DDT exposure is recent, the DDE/DDT ratio is low, whereas in areas where substantial time has passed since use, the DDE/DDT value is higher. Because DDE is attracted to fat, levels in breast milk are often six to seven times higher in a mother's milk than in her blood.

Although DDT residues in breast milk have been measured in more than 60 countries, only a few nations have comprehensive trend data where multiple studies have been done over time, using large study populations and consistent methods. After the restriction and ban of DDT in some nations, average breast-milk levels decreased substantially. Smith analyzed trend data from around the world and found that the average levels of DDT in breast milk in most countries declined in direct correlation with the length of time since DDT restriction. DDT levels in breast milk in Sweden continuously declined from 1967 through 1997. The use of DDT was severely restricted in Sweden in 1970 and completely banned in 1975. Germany has also witnessed a rapid decline in average concentrations of DDT in breast milk. Between 1969 and 1995, detectable residue levels decreased by 81%. DDT was banned in Germany in 1972. Other countries where studies have revealed a downward trend include Canada, Denmark, Norway, Switzerland, Turkey, Yugoslavia, the Czech Republic, Great Britain, Hong Kong, Israel, India, and Japan. (Solomon GM, Weiss PM).

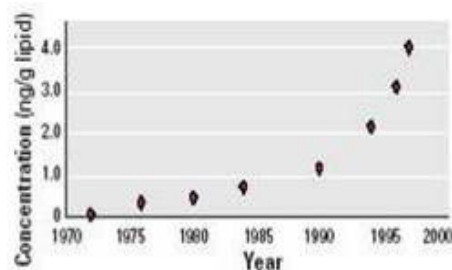
Image: Solomon GM, Weiss PM. Chemical Contaminants in Breast Milk: time trends and regional variability. *Environmental Health Perspectives*. 2002; 110(6):341. Reproduced with permission from EHP.

Biomarkers and human biomonitoring

CASE STUDY: PBDEs IN BREAST MILK - SWEDEN

Biomonitoring has shown that:

- ❖ PBDEs may affect hormone function and may be toxic to the developing brain
- ❖ Children likely to score lower on mental and physical development tests
- ❖ Key to identify previously unknown population exposure
- ❖ Key to document effectiveness of restrictions



Solomon. *Environ Health Perspectives*, 2002

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Biomonitoring of breast milk for persistent pollutants identified the rise of PBDE exposure in breast feeding women and raised concern about exposure to the fetus and breast feeding baby. The Swedish data spurred other regions to investigate PBDEs which have been identified as an increasing exposure throughout the industrialized world.

Refs:

- Herbstman, JB et al. Prenatal exposure to PBDEs and neurodevelopment. *Environmental Health Perspectives*, 2010, 118:712–719.
- Solomon GM, Weiss PM. Chemical Contaminants in Breast Milk: time trends and regional variability. *Environmental Health Perspectives*, 2002, 110(6):339-347.

Polybrominated diphenyl ethers. PBDEs are a class of widely used flame retardants. They are added to the plastic material in televisions and computers and are also found in construction materials, furniture, and textiles. Unlike the PCBs and many of the organochlorine pesticides, the PBDEs are still widely used throughout the world. The production and use of PBDEs have steadily increased since the 1970s. PBDEs can enter the environment during the production and disposal of materials containing PBDE flame retardants, as well as during the lifetime of PBDE-containing products. PBDEs are not chemically bound to plastics, so they can evaporate into indoor air or the outdoor environment. Once released, PBDEs can build up in the environment and in living organisms, binding strongly to sediment and building up in fish and other aquatic organisms.

The similarity of the PBDEs to dioxins and PCBs has been a concern because their negative effects on health may prove to be similar. In particular, scientists have found indications that the PBDEs may affect hormone function and may be toxic to the developing brain. The PBDEs have been associated with non-Hodgkin lymphoma in humans, a variety of cancers in rodents, and disruptions of thyroid hormone balance. [AS OF THE WRITING OF THIS ARTICLE] No restrictions have been placed on the production and use of PBDEs, but the Swedish government has announced an intention to ban PBDEs in products sold in Sweden, based partly on the detection of these chemicals in breast milk.

Only a few studies have sought to measure PBDEs in breast milk. Extensive data from Sweden and some limited data from Germany have been collected. In the Swedish study, archived samples collected between 1972 and 1997 were analyzed for the presence of PBDEs to get an overall summed total of PBDEs in milk. An average for each time period was calculated. The data from Sweden show a logarithmic increase in the quantity of PBDEs detected in women's breast milk. (Solomon GM, Weiss PM).

Since the Solomon article was written, Sweden did ban some PBDEs from use and levels in breast milk have begun to fall. Several other countries have also placed bans on uses and sales of certain products with individual PBDEs. Biomonitoring documenting both exposure and effectiveness of these bans is a critical link in the chain of sound environmental public health policy.

Image: Solomon GM, Weiss PM. Chemical Contaminants in Breast Milk: time trends and regional variability. *Environmental Health Perspectives*. 2002; 110(6):341. Reproduced with permission from EHP.

Biomarkers and human biomonitoring

CASE STUDY: IMPROVING INDOOR AIR QUALITY - MEXICO

- ❖ Crude wood-burning stoves used for cooking expose women and children to dangerous indoor air pollution
- ❖ 10 adults and 10 children studied before and after intervention
- ❖ Intervention: Cleaning soot, paving floor, new stove with metal chimney vent
- ❖ Biomarker proof of decreased exposure and decreased harm
 - Lowered carboxyhemoglobin (COHb) and DNA damage

	Levels of carboxyhemoglobin	
	Before risk reduction program	After risk reduction program
Mean (% COHb)	4.9*	1.0
S.D. (% COHb)	4.3	0.14
%<2.5%	45	100
%>2.5%	55	0.0
Range (%COHb)	1.05-13.88	0.65-1.30

Exposure Marker (* $p < 0.05$.)

Based on: Torres-Dorsal, 2008, *Sci Total Environ* 390: 362–368

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Biomarkers can be used to test for the efficacy of environmental interventions by measuring exposures and effects. This experiment in Mexico nicely demonstrates that, both in statistically significant reductions in carboxyhemoglobin (COHb, a measure of exposure to wood burning stove smoke) and DNA damage in lymphocytes (a measure of effect or damage from PAH and other known mutagen/carcinogens emitted by wood burning). This study was a pilot study, but the results were so dramatic and consistent, even though the sample size was very small, that the government of the state (San Luis Potosi), using the precautionary principle, decided to expand the intervention to other similar communities.

Ref:

•Torres-Dosal A et al. Indoor air pollution in a Mexican indigenous community: evaluation of risk reduction program using biomarkers of exposure and effect. *Science of the Total Environment*, 2008, 390:362–368.

Indoor air pollution can be an important risk factor for human health, considering that people spend more than 60% of their time indoors. Fifty percent of the world population and approximately 90% of the rural population in developing countries are using biomass as energy source. Latin America represents 12% of the global consumption of biomass; in Mexico, 27 million people use wood as an energy source. Therefore, in this study we evaluated a 3-stage risk reduction program. The stages were: 1) removal of indoor soot adhered to roofs and internal walls; 2) paving the dirt floors; and 3) introduction of a new wood stove with a metal chimney that expels smoke outdoors. The complete intervention program was applied. In 20 healthy subject residents from an indigenous community in San Luis Potosí, Mexico, we measured blood carboxyhemoglobin (% COHb), DNA damage (comet assay) in nucleated blood cells, and urinary 1-OHP levels before and after the program. Before intervention individuals had a geometric mean COHb level of 4.93% and 53% of the population presented levels above 2.5% considered a safe level. However, in all the studied individuals the levels of COHb were reduced to below 2.5% (mean level 1.0%) one month after the intervention. Moreover, when compared, DNA damage in people exposed before the intervention was higher (5.8 ± 1.3 of Tail Moment) than when the program was introduced (2.8 ± 0.9 of Tail Moment) ($P > 0.05$) and a same trend was observed with urinary 1-OHP levels; 6.71 ± 3.58 $\mu\text{mol/mol}$ creatinine was the concentration before intervention; whereas, 4.80 ± 3.29 $\mu\text{mol/mol}$ creatinine was the one after the program. The results suggest that the intervention program offers an acceptable risk reduction to those families that use biomass for food cooking. (Torres-Dosal A, et al.).

Image based on: Torres-Dosal A, et al. Indoor air pollution in a Mexican indigenous community: evaluation of risk reduction program using biomarkers of exposure and effect. Science of the Total Environment, 2008, 390:364.

Biomarkers and human biomonitoring

CASE STUDY: CHLORPYRIFOS, TOXICITY & SUSCEPTIBILITY

- ❖ 263 inner-city pregnant women maternal and cord blood biomonitoring
 - CPF found in 98% mothers, 94% cord blood
 - At exposures derived from standard use patterns
 - **Decreased birth weight, birth length**, statistically significant (but not clinically abnormal)
 - CPF found to be an independent determinant

Perera, 2003

- ❖ 404 inner-city infant mother pairs
 - Detectable CPF in **paraoxinase deficient mothers** was associated with small but significant **decreased head circumference in babies**

Berkowitz, 2004

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Chlorpyrifos (CPF) is an organophosphate insecticide that was so widely used after 1990 in the U.S. that it was targeted by researchers who were concerned about children's environmental health. Numerous studies documented ubiquitous exposures in all age groups as measured by urinary metabolites. CPF is known to be neurotoxic in low doses in rodents, so concern developed about prenatal exposures and their potential effects in humans. These two studies demonstrate how the use of biomarkers as part of a well-designed exposure and epidemiology study can provide the critical link between external exposure (measured by questionnaire) and abnormal effect, tying what is known from animal toxicity studies directly to human adverse effects.

Perera studied inner-city pregnant women who filled out questionnaires which correlated organophosphate exposure with maternal and cord blood measurements of CPF. Ubiquitous exposures were documented and CPF was found to be a significant independent determinant in decreasing birth weight and birth length. Berkowitz found that mothers low in paraoxinase 1 (PON1), a specific detoxifying enzyme, had babies with decreased head circumference when exposed to CPF prenatally. These are both examples of how biomarkers can be used in conjunction with environmental exposure modeling (questionnaire) to define dose response curves (correlating exposure (questionnaires) to internal dose (maternal and cord blood) and abnormal effect (decreased birth weight and birth length)). In the case of the Berkowitz study, a susceptibility biomarker for the child was the mother's defect on PON1 resulting also in a decrease in head circumference in their babies.

Refs:

•Berkowitz GS et al. *In utero* pesticide exposure, maternal paraoxonase activity, and head circumference. *Environmental Health Perspectives*, 2004, 112(3):388-391.

Although the use of pesticides in inner-city homes of the United States is of considerable magnitude, little is known about the potentially adverse health effects of such exposure. Recent animal data suggest that exposure to pesticides during pregnancy and early life may impair growth and neurodevelopment in the offspring. To investigate the relationship among prenatal pesticide exposure, paraoxonase (PON1) polymorphisms and enzyme activity, and infant growth and neurodevelopment, we are conducting a prospective, multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City. In this report we evaluate the effects of pesticide exposure on birth weight, length, head circumference, and gestational age among 404 births between May 1998 and May 2002. Pesticide exposure was assessed by a prenatal questionnaire administered to the mothers during the early third trimester as well as by analysis of maternal urinary pentachlorophenol levels and maternal metabolites of chlorpyrifos and pyrethroids. Neither the questionnaire data nor the pesticide metabolite levels were associated with any of the fetal growth indices or gestational age. However, when the level of maternal PON1 activity was taken into account, maternal levels of chlorpyrifos above the limit of detection coupled with low maternal PON1 activity were associated with a significant but small reduction in head circumference. In addition, maternal PON1 levels alone, but not PON1 genetic polymorphisms, were associated with reduced head size. Because small head size has been found to be predictive of subsequent cognitive ability, these data suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity.

•Perera FP et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. *Environmental Health Perspectives*, 2003, 111(2): 201-205.

Inner-city, minority populations are high-risk groups for adverse birth outcomes and also are more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), polycyclic aromatic hydrocarbons (PAHs), and pesticides. In a sample of 263 nonsmoking African-American and Dominican women, we evaluated the effects on birth outcomes of prenatal exposure to airborne PAHs monitored during pregnancy by personal air sampling, along with ETS estimated by plasma cotinine, and an organophosphate pesticide (OP) estimated by plasma chlorpyrifos (CPF). Plasma CPF was used as a covariate because it was the most often detected in plasma and was highly correlated with other pesticides frequently detected in plasma. Among African Americans, high prenatal exposure to PAHs was associated with lower birth weight ($p = 0.003$) and smaller head circumference ($p = 0.01$) after adjusting for potential confounders. CPF was associated with decreased birth weight and birth length overall ($p = 0.01$ and $p = 0.003$, respectively) and with lower birth weight among African Americans ($p = 0.04$) and reduced birth length in Dominicans ($p < 0.001$), and was therefore included as a covariate in the model with PAH. After controlling for CPF, relationships between PAHs and birth outcomes were essentially unchanged. In this analysis, PAHs and CPF appear to be significant independent determinants of birth outcomes. Further analyses of pesticides will be carried out. Possible explanations of the failure to find a significant effect of PAHs in the Hispanic subsample are discussed. This study provides evidence that environmental pollutants at levels currently encountered in New York City adversely affect fetal development.

Biomarkers and human biomonitoring

CASE STUDY: BODY BURDEN, POLLUTION IN NEWBORN

- ❖ 10 newborns born in U.S. hospitals in Aug-Sept 2004
- ❖ 413 industrial and consumer product chemicals tested
 - 287 found, average of 200 per cord blood sample
 - 180 carcinogens, 217 neurotoxic, 208 developmental toxicants
 - Most at levels measured in parts per trillion
- ❖ Funded privately, performed by non-governmental organization, accredited labs/technique
- ❖ Harmful or helpful?

*Industrial pollution begins in the womb
Hundreds of toxic chemicals measured
in newborn babies*



WHO

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This case study is an example of use of biomonitoring by an advocacy group. 10 newborns were selected to receive 10,000 U.S. dollars worth of chemical testing on a cord blood sample collected at the time of birth. It is of note that these 10 babies were in no way representative of any group, and that non-profits were not bound by the same ethical considerations required of most university and government research institutions. It is important to scrutinize reports that do not go through the peer review process with extra care for appropriate design, methodology and scientific rigor. Nonetheless, reports such as these often do identify important questions. In this case, how exposed are children *in utero*, and what impact might this have on their growth and development both in the womb and later in life.

Ref:

•Environmental Working Group. Body Burden – The Pollution in Newborns: A benchmark investigation of industrial chemicals, pollutants and pesticides in umbilical cord blood. EWG, 2005.

In a study spearheaded by the Environmental Working Group (EWG) in collaboration with Commonweal, researchers at two major laboratories found an average of 200 industrial chemicals and pollutants in umbilical cord blood from 10 babies born in August and September of 2004 in U.S. hospitals. Tests revealed a total of 287 chemicals in the group. The umbilical cord blood of these 10 children, collected by Red Cross after the cord was cut, harbored pesticides, consumer product ingredients, and wastes from burning coal, gasoline, and garbage.

Of the 287 chemicals we detected in umbilical cord blood, we know that 180 cause cancer in humans or animals, 217 are toxic to the brain and nervous system, and 208 cause birth defects or abnormal development in animal tests. The dangers of pre- or post-natal exposure to this complex mixture of carcinogens, developmental toxins and neurotoxins have never been studied.

Image: WHO

Biomarkers and human biomonitoring

SUMMARY

- ❖ Understanding exposure is key to understanding environmental illnesses
- ❖ Environmental monitoring coupled with exposure modeling is one approach to estimating exposures but is subject to error and uncertainty
- ❖ Biomonitoring is able to measure integrated exposures within the human body but alone cannot explain where or how the exposure occurred or the toxic potential for that exposure
- ❖ An integrated approach that uses all data types along the environmental disease continuum is required for a complete understanding of environmental illness

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<<READ SLIDE>>

<<NOTE TO USER: Add points for discussion here according to the needs of your audience.>>

Biomarkers and human biomonitoring

ACKNOWLEDGEMENTS

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