Predicting Later-Life Outcomes of Early-Life Exposures

On October 14–15, 2010, the National Academies Standing Committee on Use of Emerging Science for Environmental Health Decisions held a public workshop on the detection of later-life effects of exposure to environmental stressors, such as malnutrition or chemicals, in utero (in the womb) or soon after birth.

Participants explored the state of this rapidly evolving field of research, focusing on (1) the early developmental origins of obesity, diabetes, high blood pressure, and other metabolic diseases and (2) early exposure to arsenic and the prediction of its effects in later life. As Committee Chair William Farland, of Colorado State University, noted, the workshop also served as a forum for researchers and decision-makers in academe, the federal government, environmental groups, and industry to consider whether and how this emerging science can be used to better protect public health.

Framing the Issue

Why do some people struggle with obesity or develop such diseases as cancer or diabetes? One might speculate that interactions between people's genes and environmental factors must be responsible, but which environmental factors play a role? And how and when do their crucial interactions with genes occur?

By altering complex developmental processes, human experiences and exposures in the womb and shortly after birth can help to set the stage for a lifetime of good health—or of chronic illness. In short, explained John Rogers, of the US Environmental Protection Agency (EPA), one's susceptibility to a number of diseases may be traceable to the nutritional status of one's mother—and possibly even of one's grandmother—and to her exposure to chemicals and other stressors before and during pregnancy. Paternal characteristics before conception may also play a role and need to be further researched, added Shuk-Mei Ho, of the University of Cincinnati.

Research on the later-life consequences of early-life exposures was energized by the groundbreaking research of David Barker, who, in the mid-1980s, found a negative correlation between birthweight and the rate of death from ischemic heart disease in men.[1] The concept that stressors early in life influence later-life health outcomes became known as the Barker hypothesis and is also known as the fetal programming hypothesis or developmental origins of health and disease (DOHaD). Since then, noted Robert

> *I am absolutely convinced that what's going on early in life sets us up, good or bad, for the rest of our lives.*
>
> —**Linda Birnbaum**

Lane, of the University of Utah, and Leslie Myatt, of the University of Texas Health Science Center, we have learned that our risk for developing a number of diseases—including cardiovascular disease, diabetes, obesity, stroke, renal disease, osteoporosis, Alzheimer's disease, and cancer—may be affected by a variety of exposures during fetal or postnatal development. Many of those diseases are also associated with premature birth, which itself appears to be a result of such exposures.

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[1] Barker 1986. Weight in infancy and death from ischaemic heart disease. *Lancet* 2:577–580.

In supporting the Barker hypothesis, Lane discussed evidence from the Dutch Famine Birth Cohort Study. During the Dutch Famine of 1944–1945, energy intake dropped from 1,800 to 400–800 calories per day for about 5 months. A series of studies looked at babies exposed to famine conditions at different times during pregnancy. People exposed during the first trimester of pregnancy were more likely, as adults, to develop cardiovascular disease, high blood pressure, dyslipidemia (abnormal amounts of cholesterol or other fats in the blood), and obesity; those exposed during the second trimester were more likely to have lung or kidney disease; and those exposed during the third trimester were more likely to suffer from diabetes, depression, schizophrenia, and antisocial personality disorder. Those studies not only provided evidence that in utero stressors influence later-life outcomes but showed that *timing* is a critical factor, noted Lane.

Animal studies have also been used to detect and explore later-life effects after early-life stressors. Rogers described two categories of animal tests used for that purpose: (1) in vivo developmental toxicity tests (tests in live animals) that are used for regulatory purposes and (2) DOHaD tests. Regulatory tests for developmental toxicity, explained Rogers, include a variety of standardized study designs—including prenatal, two-generation reproduction, extended one-generation, and developmental neurotoxicity and immunotoxicity designs—that vary in the timing and duration of exposure and in the outcomes measured. DOHaD study designs are used in basic research mainly to define pathways and health outcomes rather than for regulatory purposes, continued Rogers.

Rogers noted that many DOHaD studies center on intrauterine growth restriction (IUGR; a condition in which fetal growth is abnormally low) in part to develop an animal model to explore the Barker hypothesis and associations with low birthweight. Karen Lillycrop, of the University of Southampton, and Rogers observed that studies of IUGR in animals use such treatments as globally restricted or protein-restricted maternal diets, in utero exposure to glucocorticoids (a class of anti-inflammatory and immunosuppressive hormones), and uterine artery ligation. Those studies have fairly consistently found increased blood pressure, dyslipidemia, impaired glucose tolerance, and vascular dysfunction in the offspring of treated mothers; these characteristics are similar to those of human metabolic disease, said Lillycrop. In dietary-restriction studies in rats, those results have been found even if the mothers were fed the restricted diet for only a few days either early in pregnancy or before conception.

Although participants generally agreed that animal studies are crucial in this field of research, they also raised a number of concerns about such studies. For example, Rogers explained, rodents are born at a developmentally earlier stage than humans: much of what occurs during the third trimester in humans takes place after birth in rodents. Such developmental differences affect how the findings of animal studies can be applied to humans. In addition, Jerry Heindel, of the National Institute of Environmental Health Sciences (NIEHS), noted, researchers studying animals typically do not conduct lifetime assessments, which are necessary for exploring some later-life conditions, such as neurodegenerative diseases and some cancers. And, despite good agreement between the results of animal studies and those of human studies, Heindel added, there is a need to explore common end points and mechanisms among species more systematically to improve the relevance of animal studies to humans. Farland pointed out that the use of inbred strains of laboratory animals probably reduces the relevance of animal studies for humans; this makes it difficult to understand gene–environment interactions fully, although this problem could be at least partially overcome by comparing different inbred strains. Finally, Martin Stephens, of the Humane Society of the United States, argued for more strategic use of in vitro tools (cell- or tissue-based experiments) and other alternatives to animal testing, perhaps by embracing a safety-based approach (in which it is determined what should be known about a chemical to be

reasonably sure that it will not cause serious health problems) rather than a hazard-based approach (in which one attempts to identify every possible problem that a chemical could cause).

Why: Predictive Adaptive Responses and Fetal Programming

Why should in utero experiences alter the development of a fetus, often detrimentally? According to the Barker hypothesis, Rogers and others explained, the fetus appears to use the in utero environment to predict and prepare for the postnatal environment. That is, an organism alters its developmental path to produce a phenotype (observable traits, such as characteristics of behavior, physiology, metabolism, or outward appearance) that gives it a survival or reproductive advantage in postnatal life, said Lillycrop. That process is referred to as a predictive adaptive response.

Maternal undernutrition, for example, can serve as an environmental cue—a signal that nutrients in the postnatal environment are scarce, explained Lillycrop. In response, fetal metabolism may change in a way that reduces fetal energy demands, increases its capacity for fat storage, and invests less in the development of bone and muscle mass. Those changes in phenotype improve the offspring's ability to survive in a nutrient-poor postnatal environment. But if the offspring finds itself in a nutrient-rich postnatal environment, its phenotype will not match the postnatal environment, and the infant will have an increased risk of storing fat and developing metabolic diseases. Lillycrop pointed out that the risk of obesity and diabetes in later life is also increased by early *overnutrition*. This suggests that a single genotype can give rise to multiple phenotypes and that the exact phenotype probably depends on intrauterine environmental cues.

In a related discussion, Lane highlighted the importance of understanding human capability to respond adaptively regardless of such factors as sex and timing of exposure to stressors experienced early in development. Understanding the array of, and mechanisms for, adaptive responses will enable better identification and potentially permit mitigation of early-life perturbations linked to disease development.

Rogers and Myatt observed that the placenta is central to the idea of fetal programming. The placenta plays important functional and metabolic roles during development. It not only anchors the fetus but also serves
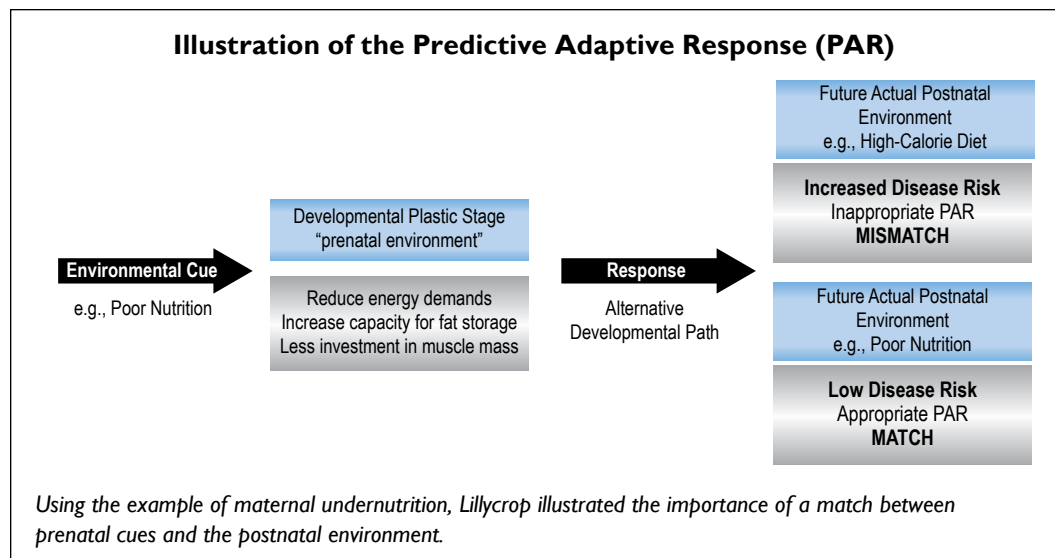
> *Is the placenta an active participant or an innocent bystander in programming? Does it just [pass on] what the mother sends to the fetus or, along the way, does it modify those nutrients and secrete other signals to affect programming?*
>
> —Leslie Myatt

as an immune barrier and a channel for gas exchange and nutrient transfer. Additionally, the placenta secretes hormones that regulate maternal metabolism and fetal growth and differentiation. The primary determinants of placental function and nutrient transport are vascular (blood flow) and trophoblast (membrane) dependent.

The placenta grows and develops throughout gestation in a carefully orchestrated manner, continued Myatt. It is highly regulated, but a number of factors can affect its pattern of growth and development and correspondingly affect fetal growth and development. First, the timing of exposure to a stressor—vis-à-vis several critical periods during development—influences how the placenta responds. Second, all imprinted genes are expressed (converted into a functional product) in the placenta, where they affect the growth of various types of placental cells. Third, placental cells express the enzyme 11ßHSD-2, which protects the fetus from the mother's high levels of cortisol (an active glucocorticoid that inhibits fetal growth) by converting it to the inactive cortisone; normal expression of 11ßHSD-2 can be interrupted by a number of stressors, including nutritional restriction.

Illustration of the Predictive Adaptive Response (PAR)

Environmental Cue
e.g., Poor Nutrition

Developmental Plastic Stage
"prenatal environment"

Reduce energy demands
Increase capacity for fat storage
Less investment in muscle mass

Response
Alternative Developmental Path

Future Actual Postnatal Environment
e.g., High-Calorie Diet

Increased Disease Risk
Inappropriate PAR
MISMATCH

Future Actual Postnatal Environment
e.g., Poor Nutrition

Low Disease Risk
Appropriate PAR
MATCH

Using the example of maternal undernutrition, Lillycrop illustrated the importance of a match between prenatal cues and the postnatal environment.

On the basis of information gleaned from the placenta, Myatt hypothesized that preeclampsia (pregnancy-induced hypertension), IUGR, and maternal obesity can affect fetal programming by causing inflammation of the placenta and by altering placental function through oxidative and nitrative stress; these processes, in turn, affect maternal–fetal metabolism and, ultimately, the health of the baby.

As the interface between mother and fetus, the placenta also holds many clues about a baby's in utero exposures, explained Myatt. In fact, he said, one could describe the placenta as a diary of what happens to a person in the womb. Rogers noted that maternal health conditions—such as diabetes and obesity, nutritional factors, toxicant exposure, stress, and infections—all determine the "maternal condition" that is transmitted to the fetus across the placenta. According to Myatt, a variety of placental characteristics in the womb and after delivery may indicate that intrauterine exposures can be measured. For example, Myatt's team found that amounts of a factor that influences the formation of blood vessels during pregnancy were dramatically lower in women with preeclampsia than in healthy controls. Perhaps, he suggested, such a measure could be used to relate placental function to outcomes. But Myatt cautioned that researchers must collect placentas only with great care because many measured characteristics may be influenced by the type of delivery (cesarean vs vaginal), gestational age, maternal medical conditions, and the baby's sex.

What Placental Characteristics Can Be Measured?

In Utero
- Blood flow and resistance to flow
- Peptide production as an index of:
  - Trophoblast invasion
  - Angiogenesis
  - Regulation of metabolism
- Steroid secretion
- Inflammatory state

Postdelivery
- Weight, size, shape, and surface area
- Histological parameters (vasculature and trophoblast structure and function)
- Vascular reactivity and compliance
- Metabolic rate
- Function (transport and peptide and steroid synthesis)
- Presence of metabolites, xenobiotics, and metals
- Redox state
- Epigenome

Myatt listed a variety of placental measurements that can be used to study fetal exposures and health outcomes.

How: Epigenetic Changes and Other Mechanisms

What is it about an in utero experience that leads to an increased risk of disease later in life? Epigenetics is key to understanding the link, said Kim Boekelheide of Brown University. Epigenetic processes produce heritable changes in gene expression, or marks, without changing the gene sequence.

Primary Epigenetic Processes
- DNA methylation: the addition of a methyl (CH_3) group to a DNA molecule
- Histone modification: changes in the proteins that help package, or "wind up," the long strands of DNA
- microRNA activity: changes in noncoding RNA involved with gene regulation

Lillycrop and Lane described epigenetic mechanisms that may be involved in the developmental origins of disease.
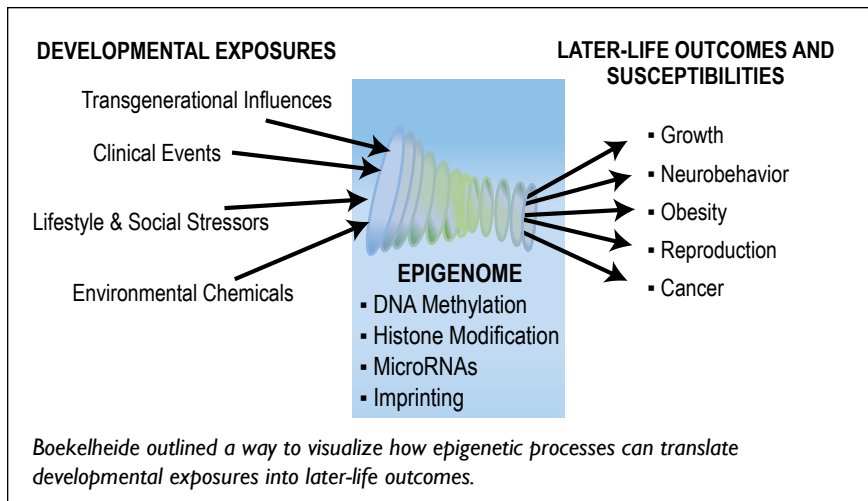
Essentially, epigenetic processes affect gene expression, "turning up" or "turning down" particular genes in particular cell types. For example, high levels of DNA methylation are usually associated with gene silencing, Lillycrop explained, whereas low levels of methylation are associated with gene activity. Many environmental exposures—including folate, cigarette smoke, heavy metals, arsenic, and depression—can influence DNA methylation patterns. Epigenetic processes constitute one way in which one's environment interacts with one's genotype to produce the phenotype, added Lane.

Lane highlighted several seminal studies that provide clues to how early-life events and exposures influence adult human health via epigenetic mechanisms:

- Folic acid supplementation of pregnant women, which is intended to reduce neural-tube defects in babies, appears to be associated with altered DNA methylation.[2]

- In vitro conception is associated with changes in DNA methylation, but with substantial variation among individuals.[3]

- DNA-methylation changes may be caused by exposure to polycyclic aromatic hydrocarbons (PAHs), components of air pollution that may

[2] Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, Heijmans BT. 2009. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. PLoS One. Nov 16;4(11):e7845.

[3] Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, Gaughan JP, Coutifaris C, Sapienza C. 2009. DNA methylation and gene expression differences in children conceived in vitro or in vivo. Human Molecular Genetics Oct 15;18(20):3769-78.

DEVELOPMENTAL EXPOSURES

- Transgenerational Influences
- Clinical Events
- Lifestyle & Social Stressors
- Environmental Chemicals

EPIGENOME
- DNA Methylation
- Histone Modification
- MicroRNAs
- Imprinting

LATER-LIFE OUTCOMES AND SUSCEPTIBILITIES
- Growth
- Neurobehavior
- Obesity
- Reproduction
- Cancer

Boekelheide outlined a way to visualize how epigenetic processes can translate developmental exposures into later-life outcomes.

> *There is certainly increasing evidence that many chronic diseases originate, at least in part, in utero, and that epigenetic processes play a key role in the developmental origins of these diseases.*
>
> —Karen Lillycrop

increase the risk of asthma. In a study of New York City children, for example, researchers found that these methylation changes were associated both with maternal reports of PAH exposure and with children's asthma symptoms before the age of 5 years.[4]

However, as several workshop participants emphasized, epigenetic mechanisms are not the only ones responsible for the later-life effects of early-life stressors. Other important mechanisms include the differentiation of stem cells in the brain, the proliferation of lung mesenchyme cells in the embryo, and alterations in the number of nephrons, the basic waste-eliminating units of the kidney. And all complex diseases develop through a gene–environment interaction, Theodore Slotkin, of Duke University Medical Center, and Heindel pointed out. Therefore, as understanding of epigenetic processes and the environmental exposures that trigger them is refined, the importance of genetic variation among individuals—and the complex interactions between genes and the environment—in the development of disease must be remembered. Slotkin added that it would be useful to know how genetic variation might mediate individual variation in resistance and susceptibility to a toxicant.

The Developmental Origins of Metabolic Disease

Several workshop participants discussed emerging evidence regarding the effects of some specific early exposures on a number of later-life outcomes, including insulin resistance, high blood pressure, and obesity.

Lillycrop's team investigated whether and how nutrition influences epigenetic processes in utero. They found that rats fed a protein-restricted diet during pregnancy produced offspring that had an increased risk of high blood pressure, dyslipidemia, insulin resistance, altered kidney structure, and cancer. Lillycrop's team also found that the offspring, as juveniles and as adults, had reduced methylation across the promoter regions (portions of DNA that control gene transcription) of the genes that code for glucocorticoid receptors (GRs) and the peroxisome proliferator–activated receptor alpha (PPARα), increased expression of the GR and PPARα genes, and an increase in the metabolic processes that the genes control. Other researchers have found that rat pups that were overfed in early postnatal life accumulated fat rapidly and were more likely to be obese and to have cardiovascular disturbances later in life; they found increased methylation of a gene that normally reduces food intake.[5] Thus, Lillycrop observed, the effects of both undernutrition and overnutrition on obesity appear to occur through the altered epigenetic regulation of genes that play key roles in metabolism and energy balance.

What about people? Can the early-life environment alter the epigenetic regulation of genes in humans? When Lillycrop's team examined the umbilical cords of normal-birthweight babies, they found that DNA methylation at specific sites at birth was strongly associated with phenotype (for example, fat mass) later in life.

Is it possible to intervene to prevent or reverse those methylation marks and disease susceptibility? Research on animals, noted Lillycrop, has shown that folic acid

[4] Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, Ho SM. 2009. Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One. 4(2):e4488.

[5] Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. 2009. Hypothalamic proopio-melanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. J Physiol. Oct 15;587(Pt 20):4963-76.

supplementation of the maternal diet can reverse the effects of protein restriction and prevent high blood pressure in the offspring. Such supplementation can also restore normal magnitudes of methylation of the genes that code for PPARα and GR and normal expression of these receptors. Lillycrop's team found similar effects from postweaning folic acid supplementation of the offspring of protein-restricted female rats. Thus, it may be possible to intervene to change methylation marks, and the peripubertal period may be an appropriate time for such intervention.

Lane and others cautioned, however, that interventions should be as specific as possible; nonspecific treatments and interventions should be considered only with a great deal of caution because "turning up" a good gene—via changes in DNA methylation, for example—may have unintended consequences. Lillycrop's work provides an example of the potential for unintended consequences of broad treatments. She found in rats that folic acid supplementation in the postweaning period results in many changes in gene expression and in phenotype. More information is needed on the effects of such changes in gene expression and on the potential unintended consequences of folic acid supplementation of pregnant women.

The Search for Obesogens

Some 68% of people in the United States are overweight and 34% are clinically obese, observed Bruce Blumberg, of the University of California, Irvine. Blumberg noted that the obesity rate in US adults, which is twice the worldwide average, had roughly doubled in the last 30 years. In 2009, obesity accounted for $147 billion in US health-care costs as a result of its association with such diseases as metabolic syndrome, a precursor of type 2 diabetes and cardiovascular disease. Obesity in pregnant women, Myatt added, is linked to such complications as diabetes, increased likelihood of cesarean delivery, prematurity, stillbirth, and large-for-gestational-age babies.

It is easy to blame obesity on what one might call couch potato syndrome—too much food and too little exercise. But Blumberg challenged that view, arguing that many other factors are involved, including one's in utero exposures.

In addition to the influence of early-life nutrition, one's likelihood of becoming obese or of developing such diseases as diabetes can also be affected by early chemical exposures. In particular, just as some substances (carcinogens) cause cancer, Blumberg's research team hypothesizes that some substances may cause obesity. He called those

> *It's pretty clear that diet and exercise are insufficient to explain this obesity epidemic, particularly in babies . . . . It's one thing to say that 50-year-old people are obese because they eat too much; it's quite another thing to say that 6-month-old babies aren't exercising enough.*
>
> —Bruce Blumberg

substances obesogens—chemicals that inappropriately stimulate adipogenesis (the formation of fat tissue) and fat storage, disturb adipose-tissue homeostasis, or alter control of appetite and lead to weight gain and obesity.

A number of hormones control appetite and metabolism, Blumberg explained. In addition, fat-cell development and lipid balance are regulated through the nuclear hormone receptors retinoid X receptor and PPAR (especially PPARγ), whose activation can increase the development of fat cells, increase fat storage in existing fat cells, and increase insulin sensitivity. Endocrine-disrupting chemicals (EDCs), which disrupt the actions of hormones or their receptors, may also be obesogens. For example, prenatal and postnatal exposure to some EDCs (such as bisphenol A, BPA) can increase weight, and several EDCs—such as phthalates, BPA, and a class of compounds called organotins—can cause undifferentiated cells to become fat cells in vitro.

Blumberg's team found that a single prenatal exposure to one organotin, tributyltin (TBT), caused permanent physiologic changes in mice, predisposing

Potential Obesogens

Environmental Toxicants
- Bisphenol A, a compound used in plastic manufacturing and for other industrial applications
- Organotins, chemicals with a variety of antifungal and other industrial uses
- Perfluorooctanoic acid, an industrial chemical used as a surfactant and for other purposes
- Phthalates, chemicals with a variety of industrial and pharmaceutical applications

Pharmaceuticals
- Antidepressants
- Atypical antipsychotics
- COX2 inhibitors, a type of nonsteroidal anti-inflammatory drug
- Diethyl stilbesterol, a synthetic estrogen formerly used to treat certain medical conditions and to minimize pregnancy loss
- Genistein, an isoflavone found in plants
- Thiazolidinedione antidiabetic drugs

Other
- Fructose
- Nicotine

Blumberg described a number of suspected or potential obesogens, including several environmental estrogens and prescription drugs.

them to gain weight as adults. TBT contains carbon-to-tin bonds and is commonly used in antifoulant paints to protect the hulls of boats and other marine structures from marine organisms. Organotins appear to induce stem cells—specifically, multipotent stromal cells—to become fat cells (rather than bone cells) and to cause existing fat cells to store more fat. The researchers then found that exposure to TBT increased the expression of the genes that code for PPARγ targets, perhaps through the undermethylation of the genes. On the basis of that and other evidence, Blumberg suggested that organotin exposure, prenatally and in adulthood, appears capable of contributing to obesity. Are people exposed to enough TBT to be of concern? The limited available data suggest that current human exposure may be sufficient to activate PPARγ.

The existence of obesogens, Blumberg emphasized, should shift the paradigm from treating obesity to preventing obesity.

The Influence of Early Exposure to Arsenic in Later-Life Outcomes

More than 140 million people worldwide are exposed to high concentrations of arsenic, mainly in drinking water, explained Michael Waalkes, of NIEHS, and Christopher States, of the University of Louisville. There are exposure hotspots in many places, including the southwestern United States and most of Southeast Asia. High levels of or chronic exposure to arsenic can induce several health outcomes, noted Joseph Graziano of Columbia University, including skin lesions, cardiovascular disease, peripheral vascular disease, lung disease, neurologic deficits, and possibly diabetes. And, Waalkes added, arsenic is also a human carcinogen at multiple sites, including the skin, urinary bladder, lung, and perhaps the liver, kidney, and prostate, despite its effectiveness as a chemotherapeutic agent that can cure some otherwise incurable leukemias.
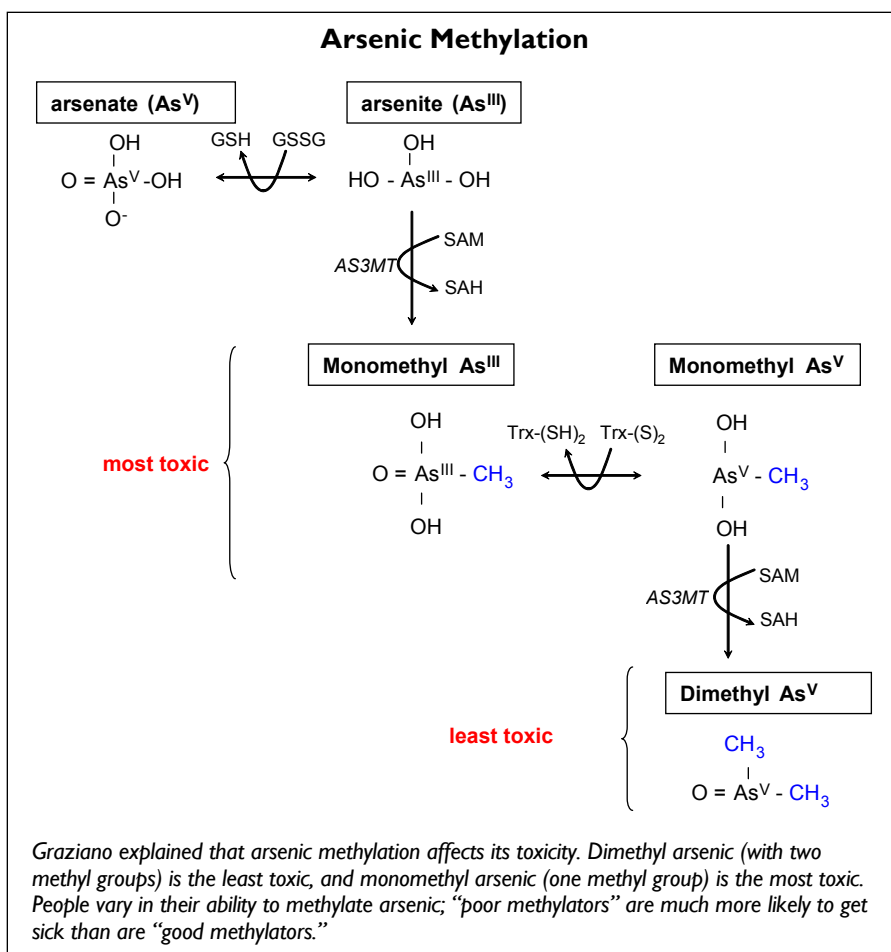
In Bangladesh, Graziano's team is studying the effects of long-term exposure of human populations to arsenic-contaminated well water as part of the Health Effects of Arsenic Longitudinal Study (HEALS). Women in that region, who have been exposed to arsenic for much of their lives, pass it on to their children, as evidenced by umbilical-cord blood arsenic concentrations that match those in maternal blood. Most of the arsenic in maternal and cord blood is in the most toxic forms, such as monomethyl arsenic. After 3 decades of arsenic exposure in the region, millions of children have been exposed, both prenatally and postnatally. Among other arsenic-induced outcomes, Graziano's team has found evidence of declines in intellectual function in the children. In the HEALS cohort as a whole, they found that cardiovascular disease is the predominant cause of arsenic-related deaths.

DNA methylation appears to play a role in arsenic-induced health outcomes. Graziano's team found that DNA methylation increases dose-dependently as a result of arsenic exposure both prenatally and in adulthood, at least when folic acid intake is adequate. Unexpectedly, however, *reduced* DNA methylation is associated with an increased risk of arsenic-induced skin lesions. Researchers are trying to reconcile the findings regarding arsenic exposure and DNA methylation.

Arsenic as a Carcinogen
Waalkes explored the mechanisms of arsenic-induced cancer by using an animal model of fetal exposure to arsenic across the placenta. As a chemotherapeutic, Waalkes said, arsenic appears to work by converting

Arsenic Methylation

Graziano explained that arsenic methylation affects its toxicity. Dimethyl arsenic (with two methyl groups) is the least toxic, and monomethyl arsenic (one methyl group) is the most toxic. People vary in their ability to methylate arsenic; "poor methylators" are much more likely to get sick than are "good methylators."

leukemic stem cells back to "normal"; thus, arsenic's carcinogenic properties may also involve stem cells. In fact, an emerging hypothesis is that cancers arise in stem-cell populations through the production of cancer stem cells. Waalkes hypothesized that the fetus may be especially susceptible to arsenic-induced cancers because of the involvement of stem cells in many of the processes that occur in utero, such as the development of internal organs and cell differentiation.

In 2004, Waalkes and his team were able to show for the first time that arsenic causes cancer in rodents, as well as in humans. Using their transplacental (TPL) model, Waalkes's team found that mice exposed in utero to arsenic (via maternal drinking water) and those exposed postnatally were more likely than control animals to develop tumors in many of the same sites in which arsenic causes tumors in humans. They also found that fetal arsenic exposure can initiate or alter tumors that are triggered later in life by exposure to estrogens and other compounds (sometimes called promoters), such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA). This apparently occurs through arsenic-induced increases in the number of stem cells: with more stem cells for TPA to target, more cancer stem cells are produced by later-life exposure. Using whole-life arsenic exposure (beginning before conception and continuing through adulthood) as a more realistic model of the type of arsenic exposure that occurs in human populations, Waalkes's team found that offspring exposed in this manner were more likely than those exposed only in utero to develop tumors in many tissue types in adulthood, and the tumors were more severe and occurred at a lower dose.

The mechanism of arsenic-induced carcinogenesis probably involves stem-cell dysfunction, aberrant DNA methylation, altered gene imprinting, or oxidative DNA damage, said Waalkes.

Arsenic and Atherosclerosis

States described research on arsenic-induced atherosclerosis (buildup of fat on artery walls), which is the root cause of cardiovascular disease. Although atherosclerosis is a disease of the arteries—causing a thickening of the arterial wall and reducing blood flow—it actually affects many organs, causing myocardial infarction (heart attack), stroke, kidney failure, peripheral arterial disease, and liver problems, such as hepatitis and cirrhosis.

To test the hypothesis that fetal arsenic exposure accelerates and worsens atherogenesis (the development of atherosclerosis), States's team used an experimental design similar to Waalkes's TPL model with a mouse
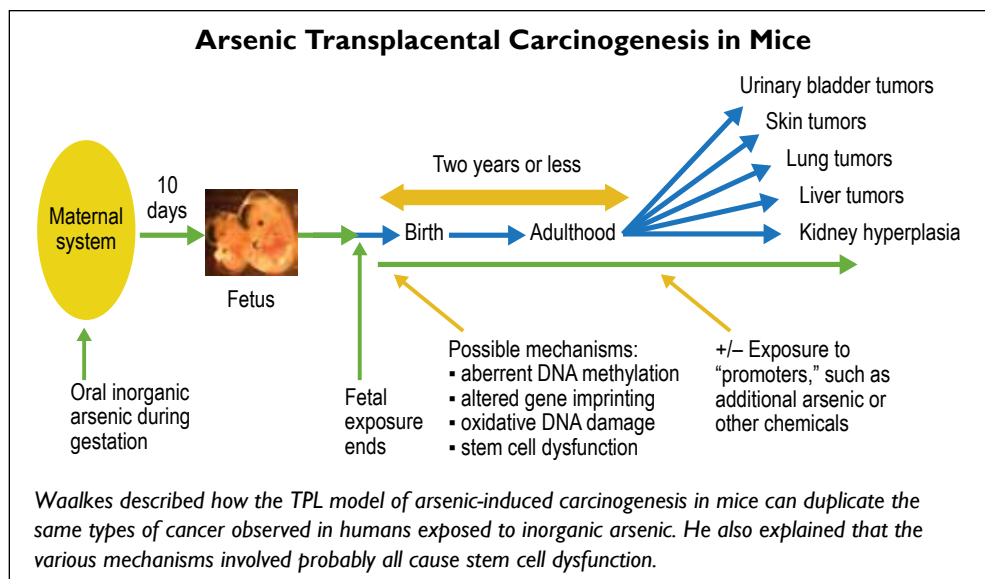
> *The disease outcome of arsenic exposure depends on the interaction of the genetics of the organism and the postnatal environment. For example, one genetic background leads to an increase in cancer, and another genetic background leads to an increase in atherosclerosis.*
>
> —Christopher States

strain that is especially susceptible to the development of atherosclerosis. In mice exposed to arsenic in utero at doses equivalent to those found in human populations with very high arsenic exposure, they found a substantial increase in lesions of the aortic arch and valve, which are indicators of atherogenesis, at the age of 10 weeks and further increases in lesion formation 6 weeks later. They concluded that in utero arsenic exposure induced an earlier onset of atherosclerosis in the mice and that the intensity of the disease increased with age. This supports the hypothesis that prenatal arsenic exposure may be atherogenic in humans.

States's team also tested the hypothesis that prenatal arsenic exposure causes liver reprogramming; this can result in a predisposition to altered inflammatory responses that contribute to atherogenesis. They found that arsenic exposure alters the developmental trajectories of mRNA and microRNA expression. In addition, they found consistent large increases

Arsenic Transplacental Carcinogenesis in Mice

Waalkes described how the TPL model of arsenic-induced carcinogenesis in mice can duplicate the same types of cancer observed in humans exposed to inorganic arsenic. He also explained that the various mechanisms involved probably all cause stem cell dysfunction.
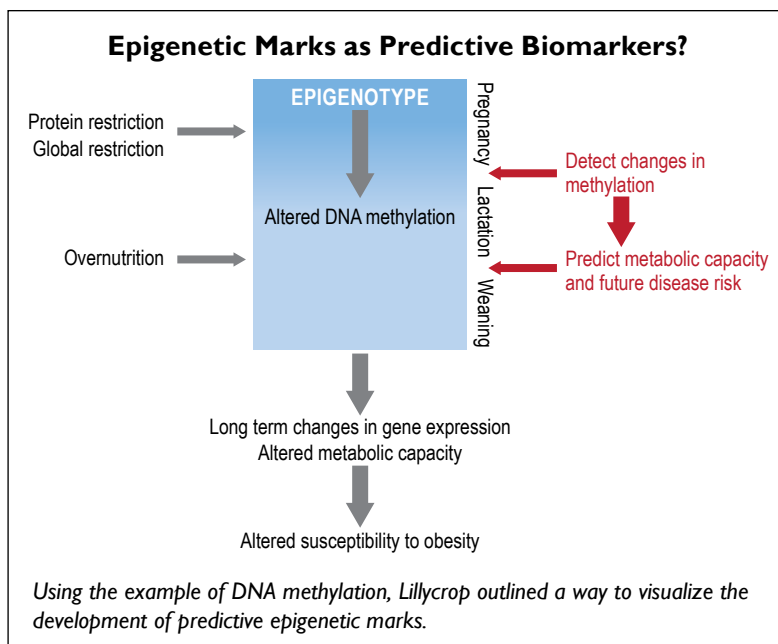
in two plasma markers of liver injury in arsenic-exposed mice, and this suggested that these mice experienced a low-grade liver injury that persisted long after the arsenic exposure. States concluded from this work that prenatal arsenic exposure alters liver development, possibly during a state of stress in early postnatal life. The liver may be primed for inflammation as arsenic-exposed animals mature, and States predicted that additional insults would cause continued liver injury and initiate atherosclerosis.

Developing Predictive Biomarkers

To use this emerging science to improve public health, researchers must find early-life biomarkers that can predict later-life disease accurately.

Some workshop participants observed that epigenetic changes, such as DNA methylation, have the potential to be useful biomarkers that predict increased or decreased risk of disease after early-life exposures. Lillycrop added that epigenetic changes may be especially useful as biomarkers because they can potentially be assessed before even the earliest signs of disease. Lillycrop cited research suggesting that methylation marks in a number of genes are stable and conserved over time and that it may eventually be possible to use various tissues, such as blood and inner-cheek samples, to detect methylation marks and predict a child's health.[6] Therefore, Lillycrop concluded, epigenetic changes could be useful as markers of future metabolic capacity and disease risk.

Speakers and panelists then mentioned a number of specific substances and processes that show promise as biomarkers. For example, States's team found evidence that circulating concentrations of the protein Hsp70 could prove to be a valuable biomarker of arsenic-induced atherosclerosis. The protein, which increases in mice after arsenic exposure and then decreases later in life, is known to induce a proinflammatory state and is associated with an increased risk of acute coronary syndrome. Graziano pointed to the potential use of brain-derived neurotrophic factor, which is affected by exposures to such toxicants as lead, as a biomarker of such exposures. Myatt suggested that some factors measurable in the placenta may prove to be useful biomarkers. For example, 11ßHSD-2 and steroid metabolism in general may be good indicators of fetal exposures. Ho and other participants suggested that small metabolites hold great promise as biomarkers. Other potential biomarkers,

Epigenetic Marks as Predictive Biomarkers?

Using the example of DNA methylation, Lillycrop outlined a way to visualize the development of predictive epigenetic marks.

said Slotkin and Bruce Fowler, of the National Center for Environmental Health, could be found in maternal and child blood samples collected as part of the National Children's Study and the National Health and Nutrition Examination Survey.

The search for biomarkers is at an early stage; ultimately, said Blumberg and other participants, there should be a plan to develop multiple-biomarker *panels,* rather than a single marker, to predict disease most accurately. In addition, States cautioned, a biomarker of exposure alone may not be sufficient; whether a disease develops later in life will probably depend on the individual's genetics and other exposures. From a practical, clinical perspective, observed Ho, the most useful biomarkers will be ones that are simple to assess (for example, with a heel stick or umbilical cord blood in infants). In addition, said Germaine Louis, of the National Institutes of Health, and others, we must better integrate epidemiology and toxicology so that early markers of later-life disease can be incorporated into epidemiologic studies.

Moving the Science Forward

Workshop participants considered several other research topics and approaches that deserve further attention. Lane emphasized the need to improve the understanding of epigenetic mechanisms before researchers work on interventions to prevent or reverse the development of disease. In particular, he asked, how do epigenetic processes result in varied responses? How do sex, tissue type, and the timing of exposure influence those responses? Future studies, Lane suggested, must take on the difficult task of tying

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6 Heijmans BT, Tobi EW, Lumey LH, Slagboom PE. 2010. The epigenome: archive of the prenatal environment. Epigenetics. 2009 Nov 16;4(8):526-31.

specific epigenetic changes to specific tissue phenotypes and functions so that it is possible to directly measure the effects of epigenetic changes on gene expression and phenotypes directly, to focus interventions to be as specific as possible, and to assess the consequences of interventions directly.

Some workshop participants observed that multiple causative events, such as exposure to particular chemicals or maternal nutritional states, can lead to a single outcome. Thus, it may be helpful to focus on a particular disease and all the stressors that can increase one's risk of that disease, rather than focusing on the effects of individual stressors. Slotkin argued that, rather than examining the most common health outcomes in the human population—including those with multiple causes—*rare* diseases should be examined as a more sensitive means of linking early causes to later effects. This approach elucidated the links between smoking and lung cancer and between maternal smoking and sudden infant death syndrome. Examining unusual outcomes that have a single cause might lead to understanding of outcomes that have multiple potential causes. Another participant pointed out that microarray technologies

> *We need to work together—industry, academe, risk assessors, and regulatory agencies—to think about what are the good data that will be useful for risk assessment, for full dose response assessment, and for understanding what is adaptive and adverse.*
>
> **– Reza Resoulpour**

have potential value in exploring multiple pathways.

Ila Cote, of EPA, and others observed that the definition of *adverse effect* requires careful consideration. For example, Cote said, one should think about whether an epigenetic change in itself (before any sign of disease) should be considered adverse. And some participants suggested that some responses to early-life exposures could be considered adaptive rather than adverse or maladaptive. For example, Slotkin suggested that high blood pressure might be adaptive. Lane argued, however, that it may not be useful to apply *adaptive* and *maladaptive* to such diseases and other developmental changes; some changes may seem adaptive initially but be maladaptive later in life.

Heindel and other participants emphasized the need for more information about the unusual dose–response relationships found in some studies in this field. For example, the effects of some EDCs and other chemicals

tend to increase with dose initially and then decrease as the dose continues to rise; this makes it impossible to extrapolate from high-dose to low-dose effects.

Reza Resoulpour, of Dow Chemical Company, and others noted that additional bioinformatics expertise will be needed to interpret the wealth of data coming from high-throughput techniques and metabolomic (the study of metabolites produced by cellular processes) research in this field. Linda Birnbaum, the Director of NIEHS, and Fowler agreed that future research on potential biomarkers and mechanisms will require the use of bioinformatics to make sense of the data and to convey findings to decision-makers and the public.

Application to Decision-Making

Current Regulatory Framework
To understand the potential use of this new science in decision-making, participants first considered current relevant laws and regulations.

EPA uses two key statutes to regulate the use of pesticides, explained Steven Bradbury, of EPA: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Under FIFRA and FFDCA, a company attempting to license a pesticide for use in the United States must provide EPA with extensive evidence of the pesticide's safety for humans, considering exposures that may occur at any time from preconception to adulthood. In such premarket evaluations, the burden of proof is on the company. EPA's job under FIFRA is to ensure that the pesticide will not cause unreasonable risk to human health or the environment, taking into account the pesticide's economic, social, and environmental costs and benefits. In addition, EPA regulates food-use pesticides (such as those used in crop production) under FIFRA and FFDCA in accordance with a risk-only standard by setting tolerances for pesticide residues in human food and animal feed, taking into account drinking water and residential exposures to ensure a "reasonable certainty of no harm".

Both statutes require EPA to consider relevant scientific information regarding pesticide safety, including developmental effects, endocrine effects, and tumor development; critical susceptibility and exposure periods; modes of action; metabolism and the potential for bioaccumulation; realistic routes of exposure; and epidemiologic data.

Challenges
What does this emerging science mean for risk assessment and regulatory decision-making? As the science evolves, Bradbury noted, EPA will need to adapt its use

of scientific information in decision-making, and it will need to do so in a focused, efficient, and transparent manner. It is important, Bradbury cautioned, to collect the right data efficiently, rather than collecting more data faster. However, pointed out Rita Schoeny of EPA, most published data are not optimal for use in risk assessment.

Cote and Bradbury offered specific guidance to help researchers to ensure that their work can be applied to risk assessment and regulation. In particular, studies must address the issues of interest to risk managers: the adverse effects that will result from exposure, the magnitude of exposure at which the effects will occur (in an exposure–dose–response framework), whether particular populations are at special risk, evidence that current test guidelines are not protective, sufficient data about the disease process to develop early diagnostic indicators for screening and reliable end points and tests, an indication of certainty regarding the link between early exposure and adult disease, and validation and acceptance of new methods used in the research. However, noted Susan Fisher, of the University of California, San Francisco, and Fowler, conducting studies that are more directly applicable for decision-making can often prove difficult because some funding agencies tend to be biased against research that

> *I would like to ask my colleagues in the risk-assessment field—particularly the regulatory folks, who just want to have a number—to embrace the complexity . . . . We [must] begin to deal with the fact that none of our simple models works particularly well when we begin to deal with the real-life scenarios.*
>
> *—Rita Schoeny*

is deemed "too practical".

Social and political factors can also pose serious barriers to the use of new scientific approaches in risk assessment and decision-making, Cote argued. Therefore, scientists must present evidence in a manner that is as convincing to the courts and to lay stakeholders as it is to other scientists; they must couple that effort with outreach to and education of other stakeholders so that they can understand and accept new science.

Opportunities

Workshop speakers and panelists discussed a number of efforts that are under way to facilitate a paradigm shift in the use of the new science in decision-making. Cote described the initiative Advancing the Next Generation

of Risk Assessment (NexGen), a multiagency effort to improve the use of molecular systems biology in risk assessment. Through this initiative, EPA and collaborating agencies will use a handful of well-studied chemicals to improve understanding of the link between molecular systems biology data and human health effects and understanding of less well-known chemicals. In addition to risk assessment, NexGen could inform alternatives assessment and hazard assessment, noted Amy Kyle, of the University of California, Berkeley. Kyle and Sara Janssen, of the Natural Resources Defense Council, suggested that NexGen might facilitate a role for EPA as an authoritative source for information for both consumers and companies on safer alternative chemicals and products. For example, information from NexGen would be helpful for California's Green Chemistry Initiative, the Consumer Product Safety Commission's Chronic Hazard Advisory Panel, and EPA's Design for Environment program.

Kristina Thayer, of NIEHS, described the use of 21st-century toxicology tools, such as the high-throughput chemical screening information being developed through Tox21, an interagency chemical research and testing effort intended to provide information that risk assessors may use in making decisions about the protection of human health and the environment. This sparked a discussion of high-throughput screening, in which Blumberg, Birnbaum, and Thayer agreed that such approaches appear to be useful for determining which chemicals are potentially hazardous but perhaps not for ensuring a chemical's safety. And, as Stan Barone of EPA cautioned, it will not be possible to test the many thousands of chemicals that it might be desirable to test; instead, the testing and evaluation process must be approached in a more intelligent, integrated manner. A January 2011 workshop organized by the National Toxicology Program addressed some of those issues.

Robert Chapin, of Pfizer, emphasized that, to move forward, the science must be able to determine which and how many early stressors cause later-life health effects and identify early markers (predictive biomarkers or biologic pathways) of later effects. Chapin then proposed three possible approaches for answering those questions and ultimately readying the new science for use in risk-assessment decision-making. The approaches differ in the time required to obtain usable information for decision-making, in expense, and in how systematically the problem is to be addressed. He noted that careful chemical selection, established performance criteria, and the use of informatics are some of the critical elements necessary for any approach. Chapin

urged participants to consider his "fast and thoughtful" approach because it should quickly provide a wealth of useful information. Some participants expressed general support for the approach. Others indicated their preference for Chapin's "build, test, and refine" approach, despite its longer timeframe and expense, or for a combination of approaches.

Is the Science Ready?

Is the understanding of the processes sufficient to inform risk assessment and other decision-making? Many workshop participants agreed that this new field of science has great potential to advance human health and well-being by allowing the prediction, and potentially prevention of, health problems. Public interest in the research is clear, Boekelheide asserted, as evidenced by a recent cover story on the topic in *Time*.[7]

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[7] Paul, AM. How the first nine months shape the rest of your life. Time 2010; October 4.

And increasingly, said Chris Weis, of NIEHS, research proposals in this field are getting the attention of funding agencies and federal government decision-makers.

Much of the science is ready—and *must* be ready—for use in decision-making, argued some participants, even if understanding is not yet perfect. Others emphasized the need to address the remaining gaps in scientific understanding of the links between early exposure and later-life disease. Nevertheless, Fowler argued, it is incumbent on the agencies responsible for risk assessment to begin to take advantage of modern scientific tools as soon as they are available and validated. Regardless of its current readiness for use in decision-making, Chapin observed, this new field of science offers the most compelling opportunity that has ever arisen to help prevent many major adult diseases.

This newsletter was prepared by Elizabeth Stallman Brown, with contributions from Keegan Sawyer, and was edited by Norman Grossblatt.

Related News and Publications

Vaidyanathan G. Prenatal exposures prompt EPA to reexamine chemical regulations. The New York Times 2010; November 18