PPTOX III: Environmental Stressors in the Developmental Origins of Disease—Evidence and Mechanisms

Thaddeus T. Schug,^{*,1} Robert Barouki,[†] Peter D. Gluckman,[‡] Philippe Grandjean,^{§¶} Mark Hanson,^{||} and Jerold J. Heindel^{*}

^{*}*Division of Extramural Research and Training, Cellular, Organ and Systems Pathobiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709;*

[†]*INSERM UMR-S 747, Université Descartes, Paris 06, 75270 France;* [‡]*Liggins Institute and National Research Centre for Growth and Development, University of Auckland, Auckland 01142, New Zealand;* [§]*Environmental Medicine, University of Southern Denmark, 5000 Odense, Denmark;* [¶]*Harvard School of Public Health, Boston, Maryland 02215; and* ^{||}*Institute of Developmental Sciences, University of Southampton, Southampton General Hospital, Southampton, Hampshire, SO16 6YD, United Kingdom*

¹To whom correspondence should be addressed at Cellular, Organs and Systems Pathobiology Branch, National Institute of Environmental Health Sciences/NIH Division of Extramural Research and Training, P.O. Box 12233, MD K3-15, Research Triangle Park, NC 27709. Fax: (919) 541-5064.

E-mail: schugt@niehs.nih.gov.

Received July 18, 2012; accepted August 28, 2012

Fetal and early postnatal development constitutes the most vulnerable time period of human life in regard to adverse effects of environmental hazards. Subtle effects during development can lead to functional deficits and increased disease risk later in life. The hypothesis stating that environmental exposures leads to altered programming and, thereby, to increased susceptibility to disease or dysfunction later in life has garnered much support from both experimental and epidemiological studies. Similar observations have been made on the long-term impact of nutritional imbalance during early development. In an effort to bridge the fields of nutritional and environmental developmental toxicity, the Society of Toxicology sponsored this work. This report summarizes novel findings in developmental toxicity as reported by select invited experts and meeting attendees. Recommendations for the application and improvement of current and future research efforts are also presented.

Key Words: developmental origins of health and disease; developmental toxicity; early-life exposure.

David Barker, in the mid-1980s, energized research on the later-life consequences of early-life exposures. He found a negative correlation between birth weight and the rate of death from ischemic heart disease in men (Barker *et al.*, 1989). This implies that normal variations in the transfer of nutrients from mothers to babies have profound long-term implications for the health of offspring. Subsequent studies showed that low birth weight was associated with an increased risk of hypertension, stroke, and type-2 diabetes.

The concept that stressors early in life influence later-life health outcomes now includes nonnutritional early-life exposures that have been shown to alter the body's physiology and is termed the developmental origins of health and disease

(DOHaD) hypothesis (Gluckman *et al.*, 2005). It is now evident that risk for developing a number of diseases—including cardiovascular disease, diabetes, obesity, stroke, renal disease, osteoporosis, Alzheimer's disease, and cancer—is affected by a variety of chemical and nutritional imbalances during fetal or postnatal development. These responses to these chemical exposures permanently change the body's structure, physiology, and metabolism. Studies in animals and epidemiological evidence strongly support the DOHaD hypothesis (Hanson *et al.*, 2008).

THE MEETING

To recognize the importance of integrating the roles that early-life nutrition and environmental exposures play in subsequent diseases later in life, the Society of Toxicology sponsored a *Contemporary Concepts in Toxicology* meeting entitled "PPTOX III: Environmental Stressors in the Developmental Origins of Disease: Evidence and Mechanisms," which was held on May 14–16, 2012 at the Espace Saint-Martin Conference Center in Paris, France. The goals of the conference were to support the DOHaD hypothesis by examining current scientific data to identify mechanisms for the effects as well as research gaps and challenges, to integrate basic and applied science, and to consider impacts on science policy and public policy.

Prior to the meeting, the organizing committee drafted a consensus paper about the current scientific insights and implications for future research and public health. [Supplementary table 1](#) lists the 11 sessions in the meeting agenda. Organized by session, this article reports on select, but not all, presentations to broadly illustrate the novel ideas and data addressed. It also provides suggestions for future research.

Session I: Opening and State-of-the-Art

Scientific evidence is necessary to impact political decisions and policy changes in developmental toxicity. Several means exist by which scientists can integrate advances made in other disciplines to advance our understanding of developmental programming. However, as Dr Ana Soto explained, scientific context is increasingly characterized by multicausality, nonlinearity, variability, and irreducible uncertainty. This complexity in turn leads to a multiplicity of legitimate perspectives and to the “manufacturing of doubt” by interests and media. Soto’s examples of lessons learned in how complexity in science impacts decision making included regulation of tobacco and the controversial evaluation of bisphenol A (BPA). She proposed that until final policy decisions are made, precautionary measures should be taken to lower human exposure well below doses causing adverse effects in animal models and behavioral changes in humans in epidemiological studies.

According to Dr Peter Gluckman, even if the science appears certain, the political process involves trade-offs against factors such as public opinion and economic, fiscal, electoral, and ideological considerations. Gluckman noted a related public policy issue is responsibility. For instance, if obesity and related diseases are believed to be caused by individual lifestyle choices, the arguments for governmental interference are reduced because of ideological constraints. But, if genetic programming is demonstrated to create a biological syndrome where behaviors or conditions are induced beyond the individual’s control, an argument for a governmental role is enhanced (Hanson *et al.*, 2011).

Providing evidence of how one scientific discipline can inform another, Dr Jerrold Heindel described the similar developmental disruptions created by environmental exposures and poor early-life nutrition. For more than 20 years, science has focused on altered programming due to poor nutrition independently from programming changes due to environmental chemical exposures. However, it is now clear that there are characteristics common to both adverse events; thus, the two fields can benefit from coordinated efforts. Both nutritional stress and environmental chemical exposures act during specific windows of exposure and demonstrate tissue-specific effects. The changes that occur are functional in nature, including alterations in gene expression, protein levels, and disruptions in the interactions between cell types that are critical to establishment of cell lineages. These changes can lead to abnormal morphological and/or functional characteristics of tissues, organs, or systems, collectively leading to disease later in life.

Session II: Programming and Epigenetics

The developmental period, when epigenetic marks undergo critical modifications, is particularly sensitive to environmental conditions (Gluckman *et al.*, 2011). Once a tissue or system is fully developed, it is less sensitive to alterations by environmental stimuli, although it may still be somewhat plastic (Barouki *et al.*, 2012). Dr Karen Lillycrop provided insight into how

environmental cues in early life, particularly maternal nutrition and behavior, induce long-term changes in an offspring’s phenotype (Burdge *et al.*, 2010). Lillycrop explained that epigenetic processes induce heritable change in gene expression without altering gene sequence. The major epigenetic mechanisms include DNA methylation, histone modification, and noncoding RNAs (Bird, 2002). A range of environmental factors, including diet, can alter the epigenome, which is most susceptible to change during the prenatal, neonatal, and pubertal periods (Burdge and Lillycrop, 2010). Epigenetic changes in a fetus induced in response to positive nutritional cues from the mother may allow development to better adapt to the future environment, whereas negative cues may result in adaptations that predispose an individual to increased risk for noncommunicable diseases (Hanson *et al.*, 2011).

A variety of physical and mental health challenges later in life may be associated with stress and exposures early in life. To address the question of mechanism leading to such challenges, Dr Moshe Szyf described the hypothesis that DNA methylation, a covalent modification of the DNA, mediates the long-term effects of early-life environmental exposure on genome function (Szyf, 2009). The pattern of distribution of methyl groups in DNA is different among various cell types and confers cell-specific identity on DNA during differentiation and organogenesis. This is an innate and highly programmed process. Szyf proposed that modulation of DNA methylation in response to environmental cues early in life serves as a mechanism of life-long genome “adaptation” that molecularly embeds the early experiences of a child (nurture) and in the genome (nature) (Szyf, 2012). He presented data supporting the hypothesis that child abuse is associated with a coordinated DNA methylation response in multiple promoters of the hippocampal glucocorticoid receptor (Labonte *et al.*, 2012).

Session III: Immune System Programming

Complex mechanistic interactions among environmental exposures, mammalian genome, and heritable lung disease are better understood through well-controlled mice studies. Such studies show that early-life exposure can modify development and function of the immune system. Dr John Hollingsworth presented evidence from mouse models, showing that dietary changes or environmental exposure to common inhaled toxicants can directly modify immune response in the lungs. Hollingsworth speculated that epigenetic programming may result in both somatic and germline inheritance of immunological phenotypes. (Boon *et al.*, 2008; Breton *et al.*, 2009, 2011; Hollingsworth *et al.*, 2008; Leng *et al.*, 2012; Li *et al.*, 2005).

Continuing the presentation of pulmonary associations, Dr Paige Lawrence explained how supplemental oxygen used to treat preterm infants with underdeveloped lungs has been linked, in survivors, with an increased risk for altered lung function and rehospitalization following respiratory infection when compared with those who were born at term (O’Reilly *et al.*, 2008). Her group discovered that adult mice given

supplemental oxygen as newborns have altered lung function that is attributed to imbalanced respiratory epithelial development. When infected with influenza A virus as adults, the mice exhibited enhanced pulmonary inflammation, morbidity, and mortality compared with infected mice that were exposed to room air at birth. Moreover, mice that recovered had pulmonary fibrosis, a pathology not typically associated with influenza virus infection. Lawrence is currently exploring mechanisms by which neonatal oxygen supplementation reprograms the host's innate and adaptive immune responses to infection (O'Reilly *et al.*, 2008; Winans *et al.*, 2011).

Upper and lower airway infections as well as reactivation of latent viral infections in humans are linked to environmental toxicants. According to Dr Carsten Heilmann, increased susceptibility to infections induced by environmental toxicants is of major importance not only at the individual level but also as a cause of increased expense to society. Heilmann explained that many studies in wildlife, laboratory animals, and humans show that exposure to environmental toxicants, predominantly in the form of organochlorine compounds, may induce an increased susceptibility to infection (Grandjean *et al.*, 2010). Heilmann tests the serum concentration of specific antibodies against vaccines as a marker of immune system dysfunction. Adverse effects were identified in children at increased exposures to polychlorinated biphenyls and perfluorinated alkylates (Grandjean *et al.*, 2012).

Session IV: Developmental Basis of Obesity/Metabolic Syndrome

Environmental exposures absorbed in the body have been found to cause side effects that either mimic or block hormones and disrupt normal metabolic functions (Diamanti-Kandarakis *et al.*, 2009). Early-life environmental exposures can be traced to subsequent risk of diseases such as type-2 diabetes and metabolic syndrome. Dr Sue Ozanne presented studies in both humans (identical twins, individuals who were *in utero* during periods of famine) and animal models that provide strong evidence for developmental programming (Martin-Gronert *et al.*, 2005), leading to a wide acceptance of the concept of the developmental origins of health and disease. Mechanisms by which chemical exposures can alter developmental programming include permanent structural changes in an organ due to exposure to suboptimal levels of essential hormones or nutrients, persistent alterations in epigenetic modifications, and permanent effects on regulation of cellular ageing through increases in oxidative stress and mitochondrial dysfunction leading to DNA damage and telomere shortening (Martin-Gronert *et al.*, 2010).

Dr Bruce Blumberg proposed the question of whether environmental factors expose preexisting genetic differences or exacerbate the root causes of diet and exercise. The environmental obesogen model proposed years ago by Blumberg stipulates that exposure to chemical "obesogens" during critical stages in development can influence subsequent adipogenesis, lipid balance, and obesity (Grun and Blumberg, 2006; Grun

et al. 2006). Tributyltin is a high-affinity agonistic ligand for both the retinoid X receptor (RXR) and peroxisome proliferator activated receptor gamma (PPAR γ). Signaling through the RXR-PPAR γ heterodimer is a key component in adipogenesis and the function of adipocytes; activation of RXR-PPAR γ can elevate adipose mass in rodents and humans (Janesick *et al.*, 2011). Blumberg discussed potential transgenerational effects of obesogen exposure and presented evidence suggesting that bisphenol A diglycidyl ether acts as an obesogen through a pathway downstream of, or parallel to, PPAR γ (Chamorro-García *et al.*, 2012).

Dr Angel Nadal presented evidence in animal and cellular models demonstrating causation between BPA exposure and higher risk of type-2 diabetes and insulin resistance. In pregnant mice, BPA exposure aggravated the insulin resistance produced during pregnancy and was associated with decreased glucose tolerance and increased plasma insulin, triglyceride, and leptin concentrations relative to controls (Alonso-Magdalena *et al.*, 2010). BPA exposure during gestation had long-term consequences for mothers: 4 months postpartum, treated females weighed more than untreated females and had higher plasma insulin, leptin, triglyceride, and glycerol levels and greater insulin resistance (Alonso-Magdalena *et al.*, 2010). At six months of age, male offspring exposed *in utero* had reduced glucose tolerance, increased insulin resistance, and altered blood parameters compared with offspring of untreated mothers, adult weight was unmodified (Alonso-Magdalena *et al.*, 2010). In addition, perinatal exposure induced weight increase (Rubin *et al.*, 2001) and glucose metabolism alteration in 6-month-old male rats (Wei *et al.*, 2011). These studies demonstrate that pregnancy may be a sensitive window for environmental exposures, for both mother and offspring.

Session V: Developmental Origins of Neurobehavioral Deficits and Disease

Brain development involves complex developmental stages that must happen at a particular time and sequence. New findings indicate that disruptions in the neurodevelopmental process can lead to permanent damage that may be reflected in cognitive deficits as well as emotional or behavioral change (Barouki *et al.*, 2012). Dr Ellen Fritsche described neurospheres, which are three-dimensional cell culture models consisting of neural progenitor cells that proliferate in culture and can migrate and differentiate into neurons and glia cells, thus mimicking *in vitro* the basic processes of brain development (Moors *et al.*, 2009). The neurosphere system is able to distinguish between positive and negative developmental neurotoxicity test compounds. Fritsche noted that comparison of effects in human and rat neurospheres reveals some species specificities [unpublished observations]. Altogether, her data show that neurospheres can be used for species-specific DNT testing in a medium-throughput way.

Dr Virginia Rauh described how prenatal exposure to chlorpyrifos (CPF), an organophosphate insecticide, is associated

with neurobehavioral deficits (Rauh *et al.*, 2011) and structural brain changes (Rauh *et al.*, 2012) in humans. The study investigated associations between CPF and brain morphology using magnetic resonance imaging in 40 children, 5.9–11.2 years of age. Twenty high-exposure children were compared with 20 low-exposure children. High CPF exposure was associated with enlargement of several brain regions. A significant CPF-by-IQ finding suggested disruption of normal IQ-surface measure associations in low CPF children. High CPF children also showed frontal and parietal cortical thinning and an inverse dose-response relationship between CPF and cortical thickness. This is the first study to report significant associations of prenatal exposure to a widely used environmental neurotoxicant, at standard usage levels, with structural changes in the developing human brain (Rauh *et al.*, 2012).

Session VI: Gene Expression, Metabolomics, and Cancer

Epigenetics may underpin interactions between genes and the environment. Environment-induced changes to the epigenome during periods of programming may contribute to increased risk of diabetes and other diseases in later life (Newnham *et al.*, 2009). Dr Toshi Shioda presented new studies on the (epi) genomic bases of xenoestrogen toxicity. Shioda developed a database of xenoestrogen dose-dependent changes in mRNA expression in the MCF-7 human breast cancer cell culture and demonstrated that transcriptomal effects of high doses of xenoestrogens are indistinguishable from effects of estradiol, whereas their effects at low doses significantly deviate from effects of the physiological estrogen (Coser *et al.*, 2003; Shioda *et al.*, 2006). In another study, xenoestrogen-induced, genome-wide changes in the mRNA expression and DNA methylation in perinatally exposed rat mammary glands were determined and compared. This “integrated genomics” approach identified several genes whose epigenetic regulations and mRNA expression are affected by xenoestrogen exposure in a coordinated fashion.

Session VII: National and Synthetic Birth Cohorts

Many epidemiological studies investigating the role of early-life exposures suffer from biases due to retrospective study designs, surrogate exposure and outcome measures, and incomplete confounder data (Vrijheid *et al.*, 2012). Pregnancy and birth cohort studies are ideally suited to improve causal inference in this field, because they are designed to study the impacts of early exposures prospectively and at multiple time points during development of the child. Furthermore, birth cohort studies usually collect biological material from mothers and children, enabling the measurement of biomarkers of exposure, early effect, or susceptibility (Valvi *et al.*, 2012; Vrijheid *et al.*, 2012).

In one such study, Dr Martine Vrijheid analyzed the effects of prenatal chemical exposure to suspected obesogens (persistent organic pollutants, BPA, and cigarette smoke) by tracking their effects over the growth trajectory from prenatal growth to

early postnatal growth and obesity in young children (unpublished data). In a Spanish birth cohort study, more than 2000 mother-child pairs were recruited in the first trimester of pregnancy (Garcia *et al.*, 2012). Vrijheid stated that DDE and HCB did not affect prenatal growth (birth weight) but was associated with rapid postnatal growth and subsequent overweight in early childhood. Associations were mainly seen in boys. Prenatal exposure to DDE, HCB, and smoking is associated with increased rapid growth and overweight risk in very young children.

Interactions between toxicants and gut microbiota can influence child health. Dr Merete Eggesbo's research team collected fecal samples for assessment of microbial colonization of the gut in 525 newborn babies, and selected microbial groups were identified by targeting small subunit microbial ribosomal RNA genes. In a subset of the babies' mothers, they also measured some common persistent environmental toxicants in human milk. His group estimated the infants' postnatal exposure to environmental toxicants throughout the first year of life by applying a modified PBPK model and studied potential associations between postnatal exposure to toxicants and the colonization process. Knowledge of the composition of a normal healthy gut microbiota during infancy is important for understanding the role of gut microbiota in disease (Eggesbo *et al.*, 2011).

Session VIII: Developmental Origins of Reproductive Diseases/Dysfunctions

During development, hormones shape the brain, and sex-specific physiology and behaviors emerge, with much of this process occurring during discrete developmental windows that span gestation through the prenatal period (Patisaul *et al.*, 2009). Perturbations during developmental windows can permanently alter the capacity for reproductive health and success.

Dr Heather Patisaul revealed that neonatal exposure to endocrine disrupting chemicals (EDCs) results in a spectrum of reproductive effects in female rodents including advanced pubertal onset and premature anovulation (Dickerson *et al.*, 2007; Gore, 2008; Mouritsen *et al.*, 2010; Patisaul *et al.*, 2010). She examined the impact of early-life, low-dose, oral exposure to EDCs, a soy phytoestrogen-rich diet, or both in combination, on reproductive maturation and ovarian morphology. Females exposed to EDCs or reared on the soy diet displayed earlier vaginal opening, a hallmark of puberty in the rat (Losa *et al.*, 2011). Soy-fed animals also had significantly more corpora lutea on postnatal day (PND 34) indicating earlier ovulation. At 5 months of age, EDC-exposed and soy-fed animals had significantly fewer corpora lutea and more cystic follicles than animals reared on the soy-free diet, regardless of EDC exposure, suggesting that these ovarian effects are not indicative of polycystic ovarian syndrome.

Dr Anders Juul explained that the timing of puberty has received considerable attention for several decades due to the

association with risk of adult diseases such as breast cancer and cardiovascular disease (Tinggaard *et al.*, 2012). A gradual decline in age at menarche has been reported in most industrialized countries. After several decades during which age at menarche appeared to be stable, data from two studies in the 1990s reported a possible new and downward trend in which age at breast development appeared to have decreased in the United States by 1–2 years. Contemporary studies now suggest similar drastic changes in European girls and to a lesser extent in European boys (Sorensen *et al.*, 2012). Earlier sexual maturation has profound implications for the clinician managing children with precocious puberty. In addition, changes of pubertal timing may be a marker of environmental changes influencing the endogenous endocrine environment at the population level.

Session IX: Developmental Basis of Cardiovascular Disease

Endogenous gonadal hormones play major roles in determining sexual dimorphism of the developing heart (Belcher *et al.*, 2012). Accumulating experimental evidence suggests a possible association between EDCs with estrogen-like activity and cardiovascular disease (Diamanti-Kandarakis *et al.*, 2009; Kendzierski *et al.*, 2012). Dr Scott Belcher presented results of studies defining the mechanisms responsible for sexually dimorphic ER-signaling in rodent cardiac myocytes (Yan *et al.*, 2011). Belcher showed that CD1 mice exposed to BPA from birth through adulthood (12–14 weeks) created alterations in cardiac function. Disruptive responses to BPA were typically estrogen-like and also observed in at least one of the EE treatment groups. In males, BPA increased left ventricular wall thickness and increased cardiac fibrosis and necrosis, without an alteration in myocyte size. In females, a significant decrease in interstitial collagen was observed (unpublished data). Belcher proposed that these cardiac pathologies result from aberrant estrogen receptor signaling caused by EDC exposure. He speculated that further mechanistic findings may pave the way for the development of therapeutic measures to protect women against arrhythmia risks associated with estrogenic chemical exposures.

Session X: Environment-Nutrition Interactions and Disease Prevention

Poor nutrition and early-life exposures share many of the same adverse characteristics that can ultimately lead to adult disease conditions. Presenters examined connections between consequences of human health in accordance with nutrition and the environment and potential methods in development for measuring life-time exposures. Dr Viet Grote discussed how postnatal breastfeeding reduces obesity at school age by about 20% compared with formula feeding. In addition to positive lifestyle and behavioral factors associated with breastfeeding, the lower protein content in breast milk compared with formula appears to be protective. High protein intakes increase the concentrations of insulin-releasing amino acids and the secretion

of IGF-1, thereby inducing an increased weight gain. In a randomized double-blind clinical trial that compared higher protein (HP) and lower protein (LP) intake with infant formula during the first year of life, Grote found significantly higher weight gain during the first year of life with a HP than with a LP formula. At 6 years of age, BMI significantly increased in the HP group and the risk for obesity increased more than two-fold compared with the LP group. Limiting the protein content of infant formulas appears to be an easy approach to reducing the risk of childhood obesity.

According to Dr Martyn Smith, “omic technologies can be applied to characterize the ‘exposome’ of specific diseases and may promote discovery of the key exposures responsible for chronic diseases” (Rappaport *et al.*, 2010). Given the importance of early-life exposures, including those occurring *in utero*, Smith stated that it is especially important to characterize the exposome of the fetus and the newborn. He proposed a “top-down” exposomic analysis of cord blood, amniotic fluid, and blood spots taken at birth and is developing techniques using these biosamples to better characterize the internal early-life exposome (Shuga *et al.*, 2010).

Session XI: Future Agenda and Conference Conclusions

Both ethical implications of developmental toxicity and future research needs in the DOHaD field should be explored. Dr Kristin Shrader-Frechette argued that scientists have ethical obligations to help prevent early-life exposures and that society should implement policies to prevent harm to current and future generations. Dr Linda Birnbaum added that while the mere presence of man-made chemicals in humans is not necessarily harmful, the increasing number of epidemiological studies showing associations between these chemicals and adverse health end points is of concern. Accidental or occupational high exposures to endocrine disruptors, industrial chemicals, pesticides, and pharmaceuticals have shown striking effects, and epidemiological and animal studies also suggest that low doses and mixtures of particular chemicals may also be unsafe, even for populations that are not typically considered vulnerable.

Studies of suspected harmful chemicals need to include doses that result in relevant internal human levels and examine a wide range of biological end points. Dose-response studies should include a range of doses to distinguish between linear and non-monotonic responses. Birnbaum and many others noted that collaborations between research scientists in academia, government, and industry should be encouraged to allow for development of more sophisticated study designs to facilitate regulatory decisions. Birnbaum emphasized that it is time to start the conversation between environmental health scientists, toxicologists, and risk assessors to determine how our understanding of low-dose effects influences the way risk assessments are performed for chemicals with endocrine-disrupting activities.

Several scientists noted that more research must be done to determine how nutritional stress and environmental chemical

exposures act during specific windows of exposure, and both demonstrate tissue-specific effects. These changes can include alterations in gene expression, protein levels, and disruptions in the interactions between cell types that are critical to establishment of cell lineages. It is also clear that there are significant epigenetic changes that appear following exposure or nutritional imbalance. Participants agreed that it is crucial to know which epigenetic changes are fundamental to disease formation and which are without consequences. This is important from a basic point of view, but also from a practical point of view as disease-relevant epigenetic changes may constitute biomarkers of clinical use.

Dr Robert Barouki challenged policy makers to take action to prevent noncommunicable diseases arising from developmental exposures to environmental chemicals or nutritional imbalances. Barouki noted that scientific evidence is now available, which was not the case a few years ago. Nutritional imbalances or exposure to certain chemicals during the prenatal period could have consequences for health later in life. Although the exact magnitude of the consequences is not known and more research is needed to determine specific mechanisms of action, existing science is ready for public action.

FUTURE RESEARCH DIRECTIONS

It is clear that many complex diseases and conditions result from a combination of genetics and environment. What is not clear is when and how this interaction of genetics and environment actually leads to disease. The DOHaD concept suggests that a wide variety of early exposures occurring during periods of time where tissues and organ systems are developing markedly increase risk for (or even cause) disease across the life course. These exposures are varied and can include drugs, nutrition, chemicals, stress, microbes, or viral infections. Potential diseases and disorders that need further investigation include obesity, type-2 diabetes, insulin resistance, asthma, cardiovascular diseases, dyslipidemia, cognitive and behavioral disorders, neurodegenerative diseases, a variety of cancers, and reproductive disorders. Evidence also suggests that disadvantaged populations may experience greater exposure to environmental hazards and exhibit higher rates of disease incidence, morbidity, and mortality. Understanding and modulating this risk in humans during critical windows of development offers the promise of disease prevention and reduction of health disparities.

Researchers should also work to identify a host of environmental stressors that increase disease risk and the mechanisms by which these exposures alter normal developmental programs and manifest in disease or conditions years and even generations later. Efforts are needed to pinpoint the unique developmental time periods in which humans are most susceptible to the combined effects of environmental exposures and genetic factors. Identifying developmental windows and discovering predictive biomarkers of exposure will dramatically increase

the ability to develop prevention strategies. Potential areas for research focus include the following:

- Develop well-characterized models and clinical research designs that promote effective multigenerational analysis.
- Identify stressors (nutritional, environmental, and social) and investigate early gene-environment interactions that may perturb the normative development of various tissues and organs.
- Apply state-of-the-art sequencing technologies to investigate epigenetic and genetic mechanisms (i.e., methylation, imprinting, and chromatin remodeling) by which early-life events lead to developmental reprogramming, impacting disease risk both in children and adults (e.g., somatic or cognitive changes) long after the stressor is gone.
- Use birth cohorts in human subjects to identify sex-specific developmental susceptibility windows for common diseases and conditions in early development.
- Identify biomarkers of developmental stress for single exposures and combinations that predict susceptibility to specific diseases and conditions later in life.
- Improve bioinformatics and statistical programs to allow the assessment and integration of developmental exposure to a variety of stresses and their importance in the development of disease outcomes.

Once it is clear how certain diseases or conditions originate in early development, effective strategies can be devised to reduce exposure to stressors and/or reduce disease incidence. Such strategies have the potential to reduce the overall societal burden of disease and alleviate health disparities.

SUPPLEMENTARY DATA

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

FUNDING

The PPTOX III meeting was funded in part by the Society of Toxicology, Agence Nationale de Securite Sanitaire Alimentation, environment, travail (ANSES), Alliance Nationale pour les Sciences de la Vie et de la Sante (Aviesan), the Society of Toxicology Endowment Fund, Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant R13HD072606, European Environment Agency, Forsythia, International Society for Developmental Origins of Health and Disease (DOHaD), National Center for Toxicological Research (NCTR), National Institute of Environmental Health Sciences (NIEHS), National Institute for Environmental Studies (NIES), Santé Environnement Toxicologie Ile-de-France, The Superfund Research Program, Université Paris Descartes, the World Health Organization, and

the Oak Foundation. The contents of this article do not necessarily represent the official views of the sponsors.

ACKNOWLEDGMENTS

The organizers of this meeting thank the Society of Toxicology for the endorsement of this event and Heidi Prange, SOT Director of Meetings, and Clarissa Russell Wilson for managing the myriad practical details associated with hosting this meeting.

REFERENCES

- Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I., and Nadal, A. (2010). Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. *Environ. Health Perspect.* **118**, 1243–1250.
- Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., and Simmonds, S. J. (1989). Weight in infancy and death from ischaemic heart disease. *Lancet* **2**, 577–580.
- Barouki, R., Gluckman, P. D., Grandjean, P., Hanson, M., and Heindel, J. J. (2012). Developmental origins of non-communicable disease: Implications for research and public health. *Environ. Health* **11**, 42.
- Belcher, S. M., Chen, Y., Yan, S., and Wang, H. S. (2012). Rapid estrogen receptor-mediated mechanisms determine the sexually dimorphic sensitivity of ventricular myocytes to 17 β -estradiol and the environmental endocrine disruptor bisphenol A. *Endocrinology* **153**, 712–720.
- Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev.* **16**, 6–21.
- Boon, K., Tomfohr, J. K., Bailey, N. W., Garantzotis, S., Li, Z., Brass, D. M., Maruoka, S., Hollingsworth, J. W., and Schwartz, D. A. (2008). Evaluating genome-wide DNA methylation changes in mice by methylation specific digital karyotyping. *BMC Genomics* **9**, 598.
- Breton, C. V., Byun, H. M., Wang, X., Salam, M. T., Siegmund, K., and Gilliland, F. D. (2011). DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. *Am. J. Respir. Crit. Care Med.* **184**, 191–197.
- Breton, C. V., Byun, H. M., Wenten, M., Pan, F., Yang, A., and Gilliland, F. D. (2009). Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *Am. J. Respir. Crit. Care Med.* **180**, 462–467.
- Burdge, G. C., and Lillycrop, K. A. (2010). Nutrition, epigenetics, and developmental plasticity: Implications for understanding human disease. *Annu. Rev. Nutr.* **30**, 315–339.
- Chamorro-García, R., Kirchner, S., Li, X., Janesick, A., Casey, S. C., Chow, C., Blumberg, B. (2012). Bisphenol A diglycidyl ether induces adipogenic differentiation of multipotent stromal stem cells through a peroxisome proliferator activated receptor gamma-independent mechanism. *Environ. Health Perspect.* **120**, 984–989.
- Coser, K. R., Chesnes, J., Hur, J., Ray, S., Isselbacher, K. J., and Shioda, T. (2003). Global analysis of ligand sensitivity of estrogen inducible and suppressible genes in MCF7/BUS breast cancer cells by DNA microarray. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13994–13999.
- Diamanti-Kandarakis, E., Bourguignon, J. P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., Zoeller, R. T., and Gore, A. C. (2009). Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocr. Rev.* **30**, 293–342.
- Dickerson, S. M., and Gore, A. C. (2007). Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. *Rev. Endocr. Metab. Disord.* **8**, 143–159.
- Eggesbø, M., Moen, B., Peddada, S., Baird, D., Rugtveit, J., Midtvedt, T., Bushel, P. R., Sekelja, M., and Rudi, K. (2011). Development of gut microbiota in infants not exposed to medical interventions. *APMIS* **119**, 17–35.
- Garcia, A. M., Gonzalez-Galarzo, M. C., Ronda, E., et al. (2012). Prevalence of exposure to occupational risks during pregnancy in Spain. *Int. J. Public Health* Jul 4 [Epub ahead print].
- Gluckman, P. D., Hanson, M. A., and Low, F. M. (2011). The role of developmental plasticity and epigenetics in human health. *Birth Defects Res. C Embryo Today* **93**, 12–18.
- Gluckman, P. D., Hanson, M. A., and Pinal, C. (2005). The developmental origins of adult disease. *Matern. Child Nutr.* **1**, 130–141.
- Gore, A. C. (2008). Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Front. Neuroendocrinol.* **29**, 358–374.
- Grandjean, P., Andersen, E. W., Budtz-Jørgensen, E., Nielsen, F., Mølbak, K., Weihe, P., and Heilmann, C. (2012). Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA* **307**, 391–397.
- Grandjean, P., Poulsen, L. K., Heilmann, C., Steuerwald, U., and Weihe, P. (2010). Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. *Environ. Health Perspect.* **118**, 1429–1433.
- Grün, F., and Blumberg, B. (2006). Environmental obesogens: Organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology* **147**(6 Suppl.), S50–S55.
- Grün, F., Watanabe, H., Zamanian, Z., Maeda, L., Arima, K., Cubacha, R., Gardiner, D. M., Kanno, J., Iguchi, T., and Blumberg, B. (2006). Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. *Mol. Endocrinol.* **20**, 2141–2155.
- Hanson, M., Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., and Gluckman, P. D. (2011). Developmental plasticity and developmental origins of non-communicable disease: Theoretical considerations and epigenetic mechanisms. *Prog. Biophys. Mol. Biol.* **106**, 272–280.
- Hanson, M. A., and Gluckman, P. D. (2008). Developmental origins of health and disease: New insights. *Basic Clin. Pharmacol. Toxicol.* **102**, 90–93.
- Hanson, M. A., and Gluckman, P. D. (2011). Developmental origins of health and disease: Moving from biological concepts to interventions and policy. *Int. J. Gynaecol. Obstet.* **115**(Suppl. 1), S3–S5.
- Hollingsworth, J. W., Maruoka, S., Boon, K., Garantzotis, S., Li, Z., Tomfohr, J., Bailey, N., Potts, E. N., Whitehead, G., Brass, D. M., et al. (2008). In utero supplementation with methyl donors enhances allergic airway disease in mice. *J. Clin. Invest.* **118**, 3462–3469.
- Janesick, A., and Blumberg, B. (2011). Endocrine disrupting chemicals and the developmental programming of adipogenesis and obesity. *Birth Defects Res. C Embryo Today* **93**, 34–50.
- Kendzioriski, J. A., Kendig, E. L., Gear, R. B., and Belcher, S. M. (2012). Strain specific induction of pyometra and differences in immune responsiveness in mice exposed to 17 α -ethinyl estradiol or the endocrine disrupting chemical bisphenol A. *Reprod. Toxicol.* **34**, 22–30.
- Labonte, B., Yerko, V., Gross, J., Mechawar, N., Meaney, M. J., Szyf, M., and Turecki, G. (2012). Differential glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide completers with a history of childhood abuse. *Biol. Psychiatry* **72**, 41–48.
- Leng, S., Stidley, C. A., Liu, Y., Edlund, C. K., Willink, R. P., Han, Y., Landi, M. T., Thun, M., Picchi, M. A., Bruse, S. E., et al. (2012). Genetic determinants for promoter hypermethylation in the lungs of smokers: A candidate gene-based study. *Cancer Res.* **72**, 707–715.
- Li, Y. F., Langholz, B., Salam, M. T., and Gilliland, F. D. (2005). Maternal and grandmaternal smoking patterns are associated with early childhood asthma. *Chest* **127**, 1232–1241.
- Losa, S. M., Todd, K. L., Sullivan, A. W., Cao, J., Mickens, J. A., and Patisaul, H. B. (2011). Neonatal exposure to genistein adversely impacts the ontogeny

- of hypothalamic kisspeptin signaling pathways and ovarian development in the peripubertal female rat. *Reprod. Toxicol.* **31**, 280–289.
- Martin-Gronert, M. S., and Ozanne, S. E. (2005). Programming of appetite and type 2 diabetes. *Early Hum. Dev.* **81**, 981–988.
- Martin-Gronert, M. S., and Ozanne, S. E. (2010). Mechanisms linking suboptimal early nutrition and increased risk of type 2 diabetes and obesity. *J. Nutr.* **140**, 662–666.
- Moors, M., Rockel, T. D., Abel, J., Cline, J. E., Gassmann, K., Schreiber, T., Schuwald, J., Weinmann, N., and Fritsche, E. (2009). Human neurospheres as three-dimensional cellular systems for developmental neurotoxicity testing. *Environ. Health Perspect.* **117**, 1131–1138.
- Mouritsen, A., Aksglaede, L., Sørensen, K., Mogensen, S. S., Leffers, H., Main, K. M., Frederiksen, H., Andersson, A. M., Skakkebaek, N. E., and Juul, A. (2010). Hypothesis: Exposure to endocrine-disrupting chemicals may interfere with timing of puberty. *Int. J. Androl.* **33**, 346–359.
- Newnam, J. P., Pennell, C. E., Lye, S. J., Rampono, J., and Challis, J. R. (2009). Early life origins of obesity. *Obstet. Gynecol. Clin. North Am.* **36**, 227–44, xii.
- O'Reilly, M. A., Marr, S. H., Yee, M., McGrath-Morrow, S. A., and Lawrence, B. P. (2008). Neonatal hyperoxia enhances the inflammatory response in adult mice infected with influenza A virus. *Am. J. Respir. Crit. Care Med.* **177**, 1103–1110.
- Patisaul, H. B., and Adewale, H. B. (2009). Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Front. Behav. Neurosci.* **3**, 10.
- Patisaul, H. B., and Jefferson, W. (2010). The pros and cons of phytoestrogens. *Front. Neuroendocrinol.* **31**, 400–419.
- Rappaport, S. M., and Smith, M. T. (2010). Epidemiology. Environment and disease risks. *Science* **330**, 460–461.
- Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., and Whyatt, R. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ. Health Perspect.* **119**, 1196–1201.
- Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., Liu, J., Barr, D. B., Slotkin, T. A., and Peterson, B. S. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7871–7876.
- Rubin, B. S., Murray, M. K., Damassa, D. A., King, J. C., and Soto, A. M. (2001). Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ. Health Perspect.* **109**, 675–680.
- Shioda, T., Chesnes, J., Coser, K. R., Zou, L., Hur, J., Dean, K. L., Sonnenschein, C., Soto, A. M., and Isselbacher, K. J. (2006). Importance of dosage standardization for interpreting transcriptomal signature profiles: Evidence from studies of xenoestrogens. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12033–12038.
- Shuga, J., Zeng, Y., Novak, R., Mathies, R. A., Hainaut, P., and Smith, M. T. (2010). Selected technologies for measuring acquired genetic damage in humans. *Environ. Mol. Mutagen.* **51**, 851–870.
- Sørensen, K., Mouritsen, A., Aksglaede, L., Hagen, C. P., Mogensen, S. S., and Juul, A. (2012). Recent secular trends in pubertal timing: Implications for evaluation and diagnosis of precocious puberty. *Horm. Res. Paediatr.* **77**, 137–145.
- Szyf, M. (2009). The early life environment and the epigenome. *Biochim. Biophys. Acta* **1790**, 878–885.
- Szyf, M. (2012). The early-life social environment and DNA methylation. *Clin. Genet.* **81**, 341–349.
- Tinggaard, J., Mieritz, M. G., Sørensen, K., Mouritsen, A., Hagen, C. P., Aksglaede, L., Wohlfahrt-Veje, C., and Juul, A. (2012). The physiology and timing of male puberty. *Curr. Opin. Endocrinol. Diabetes. Obes.* **19**, 197–203.
- Valvi, D., Mendez, M. A., Martinez, D., Grimalt, J. O., Torrent, M., Sunyer, J., and Vrijheid, M. (2012). Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: A prospective birth cohort study. *Environ. Health Perspect.* **120**, 451–457.
- Vrijheid, M., Casas, M., Bergström, A., Carmichael, A., Cordier, S., Eggesbø, M., Eller, E., Fantini, M. P., Fernández, M. F., Fernández-Somoano, A., et al. (2012). European birth cohorts for environmental health research. *Environ. Health Perspect.* **120**, 29–37.
- Wei, J., Lin, Y., Li, Y., et al. (2011). Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. *Endocrinology* **152**, 3049–3061.
- Winans, B., Humble, M. C., and Lawrence, B. P. (2011). Environmental toxicants and the developing immune system: A missing link in the global battle against infectious disease? *Reprod. Toxicol.* **31**, 327–336.
- Yan, S., Chen, Y., Dong, M., Song, W., Belcher, S. M., Wang, H. S. (2011). Bisphenol A and 17beta-estradiol promote arrhythmia in the female heart via alteration of calcium handling. *PLOS ONE* **6**, e25455.