OBTAINING EXPOSURE ASSESSMENT DATA AND ITS APPLICATION TO DISEASE ETIOLOGY

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Centuries ago, Paracelsus taught us that the dose (of a chemical) makes the poison. Since that time we have amended that phrase (at least for some health endpoints) to read the dose at critical windows (susceptibility windows) of development makes the poison. While most scientific people in our field agree with this premise, there is spirited debate about both the magnitude of the dose of a given chemical needed to cause a given disease and the window of highest susceptibility for the dose of that chemical to cause that disease. For example, even diseases that manifest themselves in adulthood have been linked to environmental insults that occurred *in utero*, postnatally, and peripuberty. No chemical/chemical family has been the subject of debate in environmental health more than dioxin and related chemicals. To establish an unequivocal causal link between dioxin and human disease, one must of course link exposure (dose) to dioxin and human disease. In the area of assessing exposure to the general population, we have credible data on the internal dose levels in various general populations at various age groups. We also have a great deal of information on its pharmacokinetics- absorption, distribution, metabolism, and elimination. Mechanistically, we have an excellent understanding of its mode of action. Given all of this, it is still very difficult to establish an unequivocal link between dioxin exposure and human disease in the general population. Most of the problem lies not on the exposure side of the paradigm but on the susceptibility and effect sides because many of the toxic effects attributed to dioxin- both cancer and noncancer- are multifactorial, difficult to diagnose and quantify, non-specific to a given cause, and may have a long latency period. As pointed out, resolving these issues will require a combination of improved diagnostic tests, large epidemiological studies, and the development of genetically manipulated animal models in which the role of cross-talk between the AhR and its receptors (such as estrogen) signally cascades can be distinguished from their individual pathways.[1,2]

However, dioxin is not unique for the biochemical and health effects to the general population resulting from exposure to many other environmental chemicals are being debated as well. It is of utmost importance that we provide the best data possible on exposure, susceptibility, and the effect portions of the paradigm. Our laboratory is very much involved in exposure assessment using biomonitoring. We are obtaining exposure information by a variety of means (such as The Center for Disease Control and Prevention's National Report on Human Exposure to Environmental Chemicals[3]) and planning for obtaining (via the U.S. National Children's Study[4]) exposure data on the general population of the U.S. The results from the National Report are based on urine,

whole blood, and serum samples collected as part of the National Health and Nutrition Examination Survey (NHANES) [5], which is administered by the National Center for Health Statistics of the CDC. NHANES is a continuous annual survey that enrolls about 5000 participants annually from 15 locations throughout the U.S. It is designed to be a stratified, complex, multistage probability sample of the civilian, noninstitutionalized U.S. population; therefore, the estimates are probability based for this segment of the population. In 1999-2000 the survey oversampled African Americans, Mexican Americans, adolescents (12-19 years), older Americans (≥ 60 years) and pregnant women; in 2000 low income whites were also oversampled. The concentration levels are presented for the entire subset population and also by age group, sex, and race/ethnicity. For these analyses, race/ethnicity is categorized as non-Hispanic black, Mexican American, and non-Hispanic white. Other racial/ethnic groups are included in estimates that are based on the entire sample population. The National Report presents tables of descriptive statistics on the distribution of blood and urine levels for each analyte. Statistics include geometric means and percentiles with confidence intervals. Exposure to selected metals, phthalates, polyaromatic hydrocarbons, phytoestrogens, and contemporary pesticides was assessed by urine analysis from random subsets of participants aged 6 years and older. Serum cotinine levels, as a biomarker of nicotine exposure, was measured in all participants aged 3 years and older. Blood lead and cadmium levels were measured in all participants aged 1 year and older. Total blood mercury was measured in children aged 1-5 years and women aged 16-49 years. Dioxins, furans, PCBs, and organochlorine pesticides were measured in serum from a random one-third sample of participants aged 12 years and older; unfortunately, only about 5 mL of serum was available for this analysis. Therefore, this measurement suffers from a large number of nondetectable values and also from not representing all age groups; e.g., perinatal and less than 12 years of age. In the 2001-2002 samples, we are attempting to get mean levels of the dioxin and furan congeners by pooling portions of serum samples from various segments of the population aged 12 years and older. In addition, we will continue to measure these levels in individual samples using an increased volume of serum. Future results from the NHANES samples will include exposure data on many other chemicals, including brominated flame retardants, perfluorinated chemicals, bisphenol A, alkyl phenols, additional metals, additional pesticides, additional PAHs, volatile organic chemicals, and acylamide.

In addition to the NHANES, we are very much involved in planning chemical exposure assessment for the National Children's Study, which has as a goal to follow 100,000 children prospectively for 20 years. The primary health outcomes that will be examined include asthma and neurodevelopmental effects. This study will bring on unique challenges in that exposure will need to be assessed to these participants throughout many stages of life, including *in utero*, infancy, young toddler, older toddler, and onto adulthood. Normative data from NHANES data for the persistent chemicals, such as dioxin, will be of limited benefit because the NHANES will provide data on only those 12 years of age and older. Also, we must also realize that biomonitoring has some disadvantages and other methods of exposure assessment will have to be used as well. These disadvantages include: sample collection may be invasive; usually biomonitoring does not identify source/pathway for the chemical to humans- thus, it may be of limited

use in risk management; analyte may not be specific for environmental chemical; interpreting biomonitoring data requires additional development (*vide infra*). So, in addition to biomonitoring we will have to use other methods for assessing human exposure to various chemicals at all life cycle stages. We will have to incorporate questionnaire and historical information, global information system data, and environmental monitoring data. We will have to combine these approaches for exposure assessment with calibrated and validated models for all age groups and other anthropomorphic differences. Nonetheless, biomonitoring data will be heavily used in epidemiological studies because this is generally the most accurate means to link exposure to health outcomes. We need increased ability to relate biomonitoring data to genetic information to better understand the progression of disease associated with environmental exposures.

Within the biomonitoring area alone there is information needed as to what is the best matrix for monitoring exposure to certain chemicals during various life stages. For example, research is needed for determining the best matrix for measuring environmental chemicals during the *in utero* or fetal period. Maternal levels, meconium, amniotic fluid, cord blood, blood spot, umbilical cord, hair/nails, and vernix have been used to assess exposure during this stage. Each of these matrices of course has advantages and disadvantages. Likewise, for the older populations, we will have to develop methods that use decidedly less matrix, such as blood, and/or develop methods for measuring environmental chemicals in biological matrices such as saliva, milk, feces, hair/nails, breath, and adipose tissue. Some methods exist for measuring selected environmental chemicals in these matrices. When using these alternative matrices, we need to validate them. For example, more work needs to be done to better understand the partitioning of environmental chemicals between matrices; e.g., milk and serum.[6]

Once we acquire all of this biomonitoring data, the question then becomes, "how do we interpret it." There is no doubt that these data contribute to an understanding of exposure and the uptake of these chemicals into the body. However, these data need to be used in a much larger framework including risk assessment. Biomonitoring data need to be modeled both backward (for example, by developing and using pharmacologically based pharmacokinetic models) in the exposure paradigm so that biomonitoring data can be tied directly to exposure information but also forward towards the effect portion of the paradigm. One area of research that will aid the latter is the development and application of genomic and proteomic tools to evaluate exposures to individuals and populations. For example, after an exposure the measurement of gene expression (either activated or suppressed) using gene chip arrays may not only document exposure to environmental agents, but may also provide guidance on differences in individual responses at the molecular level.[7] If biomarkers showing protein expression (proteomics) can be developed, this will indicate how the body responds to environmental insults and the biological mechanisms of how the body's defense systems and metabolism of environmental contaminants are proceeding. These will be real challenges but will aid in preventing environmentally related diseases.[8]

References

1. Ohtake, F., Takeyama, K-I., Matsumoto, T., Kitagawa, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanaglsawa, J., Yoshiaki, F.K., and Kato, S. 2003. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. *Nature* **423**:545-550.

2. Brosens, J.J. and Parker, M.G. 2003. Oestrogen receptor hijacked. *Nature* **423**: 487-488.

3. Centers for Disease Control and Prevention. Second National Report on Human Exposure to Environmental Chemicals. Available at: http://www.cdc.gov/exposurereport/ Accessed July 14, 2003.

4. Branum, A.M., Collman, G.W., and the National Children's Study Interagency Coordinating Committee. 2003. The National Children's Study of Environmental Effects on Child Health and Development. *Environ Health Perspect* **111**:642-646.

5. National Center for Health Statistics. National Health and Nutrition Examination Survey (NHANES). Available at: http://www.cdc.gov/nchs/nhanes.htm Accessed July 14, 2003.

6. Aylward, L.L., Hays, S.M., LaKind, J.S., and Ryan, J.J. 2003. Rapid communication: Partitioning of persistent lipohilic compounds, including dioxins, between human milk lipid and blood lipid: an initial assessment. *J. Toxicol. Environ. Health, Part A* **66**:1-5.

7. Mori, C., Komiyama, M., Adachi, T., Sakurai, K., Nishimura, D., Takashima, K., and Todaka, E. 2003. Application of toxicogenomic analysis to risk assessment of delayed long-term effects of multiple chemicals, including endocrine disruptors in human fetuses. *Environ Health Perspect* **111**: 803-809.

8. Lioy, P. Biomarkers- an exposurologist views their continued integration with environmental health science. *Risk Policy Report*-(February 18, 2003) 35-37. Available at: http://www.InsideEPA.com.