What do we understand about children's susceptibility for certain environmentally related disease outcomes?

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LTG 3 Poster 02

Science Questions

- What genetic and physiological factors can be used to better characterize age groups in terms of susceptibility to environmentally related disease?
- How can PBPK modeling be used to better characterize susceptible age groups based on differential dosimetry?
- Which environmentally related disease mechanisms are unique to specific age-related groups (e.g. children ages 0-2 years old)?

Research Goals

Recent environmental legislation has specified the assessment of risks to infants and children as part of regulatory decision making. In order to effectively assess these risks the Agency must characterize the vulnerability of specific age groups to the effects of environmental hazards. There is much evidence to suggest that infants and children are both more susceptible and more highly exposed to environmental hazards such as pesticides, heavy metals, polycyclic organics, particulate matter and volatile organic chemicals. This research is particularly important in understanding the vulnerability of infants and children to the environmental exposures that may contribute to diseases such as autism, other neurobehavioral deficits and asthma.

Results & Conclusions

Genetic Susceptibility

- Newborns appear to have lower levels of PON1 than adults (Figure 2) and increase post-partum, reaching a plateau between 6 and 24 months of age (Figure 3).
- Other SNPs (besides PON1192QR) also appear to influence the variability due to both enzyme activity and expression.
- Combining information on PON1 genotype and phenotype is essential in order to identify individuals who are vulnerable to pesticide exposure.
- Children less than 2 years of age represent a particularly vulnerable population to pesticide exposures.

PBPK Modeling

- PBPK modeling has demonstrated the feasibility of integrating anatomic, physiologic and biochemical variance to assess their separate impact on the formation of a risk-relevant pharmacokinetic outcome (Figure 4).
- Research indicates that physiological differences may play a more important role than physico-chemical or biochemical factors in determining tissue dosimetry (Figure 5).

Health Outcomes & Susuceptibility

- *In utero* exposure to organophosphates (OPs) are linked to abnormal infant reflexes, a critical marker of neurologic integrity.
- Children whose mothers have a specific PON1 genotype had significantly lower mental development as measured by the Bayley's Scale (Figure 7).
- There are gene expression differences in peripheral blood in children with autism compared to the general population.
- Two new animal models have been developed for assessment of neurotoxicity of environmental agents.
- Prenatal exposure to PCBs may affect growth, especially in girls, and *ortho*-substitution is an important determinant of its effect on growth (Figure 8).
- Epidemiologic studies have revealed a relationship between environmental exposure to polycyclic organic compounds and deficits in prenatal growth and development.

Methods & Approaches

Genetic Susceptibility: Paraoxonase (PON) 1 is an important enzyme in the detoxification of organophosphahate (OP) pesticides (Figure 1) which exhibits considerable variation among individuals due to genetic polymorphisms that alters its activity and to differences in plasma levels of the enzyme. Of particular importance is a single nucleotide polymorphism (SNP) in the coding region (PON1Q192R) that expresses two forms of the enzyme with differing levels of activity. Recent ORD sponsored research has combined mechanistic and genetic studies with information from birth cohorts to further characterize the real and potential vulnerability of children to pesticide exposures.

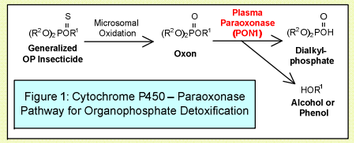

Figure 1: Cytochrome P450 – Paraoxonase Pathway for Organophosphate Detoxification

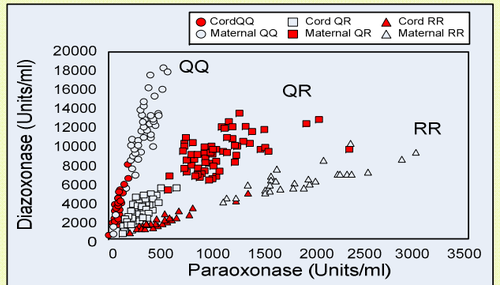

Figure 2: PON1 status plot for mother (maternal blood) and newborns (cord blood) determined by two substrate analysis. Plot clearly separates the PON1Q192 homozygotes, the heterozygotes and PON1R192 homozygotes for each group (Furlong et al., 2006).

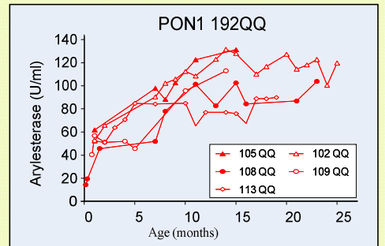

Figure 3: Developmental Onset of Plasma PON1 Activity in Individual Infants. The time course of the appearance of PON1 was variable among individuals, with PON1 levels reaching a plateau as early 5 months of age and as late as 2 years. PON1 activity determined by phenylacetate hydrolysis assay. Each line represents the time course of PON1

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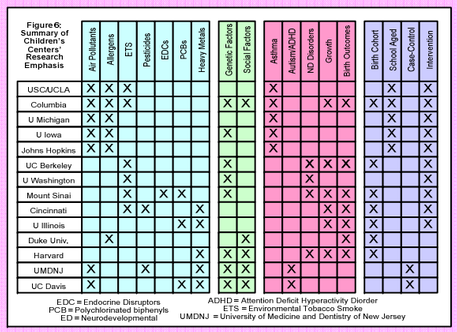

Figure 6: Summary of Children's Centers' Research Emphasis

	Air Pollutants	Allergens	ETS	Pesticides	EDCs	PCBs	Heavy Metals	Genetic Factors	Social Factors	Asthma	Autism/ADHD	ND Disorders	Growth	Birth Outcomes	Birth Cohort	School Aged	Case-Control	Intervention
USC/UCLA	X	X	X							X						X		X
Columbia	X	X	X					X	X	X			X	X	X	X		X
U Michigan	X	X								X						X		X
U Iowa	X	X						X		X						X		X
Johns Hopkins	X	X								X						X		X
UC Berkeley			X					X				X	X	X	X			X
U Washington			X					X				X						X
Mount Sinai			X		X	X		X				X	X	X	X			X
Cincinnati			X	X			X						X	X	X			X
U Illinois						X	X						X	X	X			X
Duke Univ.		X							X					X	X			
Harvard							X	X	X			X	X	X	X			
UMDNJ	X			X			X	X	X		X					X		
UC Davis	X					X	X	X	X		X						X	

EDC = Endocrine Disruptors
PCB = Polychlorinated biphenyls
ED = Neurodevelopmental
ADHD = Attention Deficit Hyperactivity Dierder
ETS = Environmental Tobacco Smoke
UMDNJ = University of Medicine and Dentistry of New Jersey

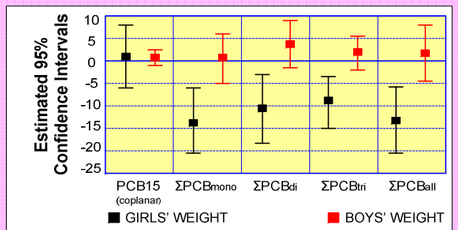

Figure 8: Estimated coefficients (95%CI) for regression models with repeated measures: birth and 4, 7, & 17 year of age of weight versus prenatal PCB exposure. ΣPCB_{all}, sum of all PCBs; ΣPCB_{mono}, sum of mono-*ortho* substituted PCBs; ΣPCB_{di}, sum of di-*ortho* substituted PCBs; ΣPCB_{tri}, of tri-*orthro* substituted PCBs; $PCB15_{(coplanar)}$, non dioxin-like, non–*ortho* substituted PCB (From: Lamb et al, *Environ.* 114 5 779-785, 2006

Pharmacokinetic Modeling: A better understanding of the differences in tissue dosimetry among human of different ages will significantly reduce the uncertainty associated with assessments of children's environmental health risk. This increased level of understanding is often brought about though the development and application of physiologically-based pharmacokinetic (PBPK) models. In order to develop such models for children, it is important to understand age-related differences in physiology and biochemistry and how interactions between these factors are indicative of age-dependent susceptibility.

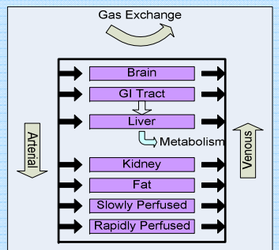

Figure 4: PBPK Model used in the analysis of age-dependent pharmacokinetics of different volitile organic compounds in the rat (Rodriguez et al, 2007).

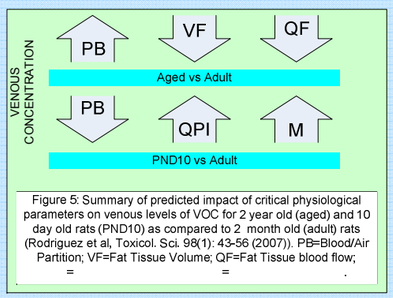

Figure 5: Summary of predicted impact of critical physiological parameters on venous levels of VOC for 2 year old (aged) and 10 day old rats (PND10) as compared to 2 month old (adult) rats (Rodriguez et al, Toxicol. Sci. 98(1): 43-56 (2007)). PB=Blood/Air Partition; VF=Fat Tissue Volume; QF=Fat Tissue blood flow;

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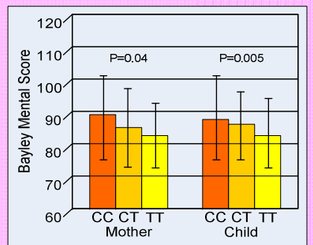

Figure 7: Mean Bayley mental scores of 2-year old children by $PON1_{108CT}$ genotype. Both maternal and child genotypes were significantly associated with child's Bayley MDI at 2 .

Health Outcomes & Susceptibility: Crucial to EPA's research efforts in this area are epidemiological studies being conducted by the joint EPA/NIEHS Centers of Excellence for Children's Environmental Health and Disease Prevention Research. These Centers employ an integrated approach combining exposure-related surveys and measurements, mechanistic studies and epidemiology in an attempt to elucidate the factors influencing childhood susceptibility to environmentally related disease. Figure 6 summarizes the main emphases of these Centers. The main thrust of the Center's research related to susceptibility and vulnerability is the use of epidemiological information to identify and characterize of populations that may be more vulnerable to environmental exposures.

Impacts and Outcomes

- **Information on genotype and phenotype can characterizes vulnerable populations.**
- **The genetic susceptibility of certain age/ethic groups may make the FQPA's 10-fold default assumption ineffective for particular populations.**
- **PBPK modeling can be used to develop uncertainty factors that can more accurately replace the default factors that are typically used to describe inter-individual variability in toxicokinetics used in environmental decision-making.**
- **Physiological factors in developing rodents will promote consistency in the PBPK modeling used in EPA decision-making and will guide researchers and modelers as they set priorities for more cost effective model development and employment.**
- **Analysis of human outcome/environment relationships can identify populations requiring special treatment or analysis in EPA risk assessments.**

Future Research

- Intitiate studies to develop generic PBPK modeling systems for infants and children that can be used by risk assessors to develop age specific dosimetry.
- Explore the impacts of single nucleotide polymorphisms other than 192QR on PON1 status.
- Continue to investigate the relationships between environmental exposures and neurobehavioral health outcomes.
- Apply knowledge of PON1 genetic variability to cumulative risk models for PO pesticides and compare the results to the 10x factor for specified for infants and children.

Contributing Researchers

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Susceptible Populations

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