CLARITY-BPA: A Novel Approach to Studying Endocrine Disrupting Chemicals

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Background

- **What is Bisphenol A (BPA)?**
  - Chemical widely used to make polycarbonate plastics & epoxy resins.
- **Where is BPA found?**
  - In food & drink packaging (water and baby bottles), compact discs, impact-resistant safety equipment, and some medical devices including those used in hospitals; epoxy resins used to coat metal products (food cans, bottle tops, & water supply pipes); also found in certain thermal paper products (cash register and ATM receipts); some dental sealants and composites.
- **How does exposure occur?**
  - Leaches into foods especially if containers are heated or washed with harsh detergents; other routes of exposure also (dermal, inhalation etc.).
- **Why the concern?**
  - Widespread human exposure from use and environmental occurrence (CDC reported BPA in 93% of Americans 6 years & older);
  - Extensive data base of animal studies reporting reproductive and developmental effects following exposure to low doses.

NIEHS and NTP Studies Address BPA Research

- By 2008 – NIEHS had funded 39 investigator-initiated grants
- In 2009 – NIEHS:
  - In-house research
  - Awarded 10 Grand Opportunity and 3 Challenge Grants under the ARRA program
  - Created the BPA Grantee consortium of > 40 researchers which meet in person yearly and by conference call once per month.
  - Publications to date: *(http://www.niehs.nih.gov/news/sya/sya-bpa/bpa-related/index.cfm)*
- Additional NTP research activities:
  - Human clinical pharmacokinetic studies; round robin assessment of BPA in serum;
  - Exposures from dental materials and thermal receipts
  - Occupational exposure assessments by NIOSH supported by NTP
  - Rodent and monkey kinetic studies at NCTR supported by NTP
  - Subchronic (90-day) perinatal exposure studies in Sprague Dawley (SD) rats at NCTR; supported by NTP

An Unprecedented Research Collaboration

- Consortium Linking Academic and Regulatory Insights on the Toxicity of BPA (**CLARITY-BPA**) was established in 2009.
- CLARITY-BPA brings together academic researchers with federal regulators to answer critical research questions. It involves scientists from:

  - NIEHS NTP and Extramural/Training program
  - FDA NCTR and CFSAN
  - 13 NIEHS-funded academic grantees (selected through a competitive review process (experts in various disease endpoints such as behavior/neuroendo, reprod., immune, obesity/diabetes, cardiovas., cancer).

- New collaborative research model that draws upon the strengths of academic and guideline-compliant research.
- Goals are to help resolve scientific uncertainties about BPA toxicity, optimize BPA-focused research investments, and to generate additional data for risk assessment.
- Ultimately, show a new way to conduct research studies by using consortium-based science.

CLARITY-BPA Research Program Organization

- Steering Committee- government and academic investigators
  - Each principal investigator (grantee) serves on the steering committee.
  - Input into design, performance, and reporting of data.
  - Monitors progress, and recommends redirection, if needed.
- Articles of Collaboration established specific responsibilities of the government (NTP and NCTR/FDA) and the grantees.
- All animals were bred, housed and treated at NCTR under a core-GLP compliant study design. Tissue samples were provided to investigators from this core study; samples were blinded as to treatment; code broken after submission of raw data to NTP data repository. Investigators are responsible for analyzing & reporting their own data.
- Investigators may publish independently, but all data will be shared and available for integrated assessments.
- External advisory scientific panel.

CLARITY-BPA Study Design

- BPA was orally administered to pregnant Sprague Dawley dams starting on gestation day 6 and continuing until start of delivery. Then, neonates were directly dosed by gavage daily until weaning on postnatal day 21. Some pups continued daily dosing (continuous dosing), while others were not further dosed (stop dose);
- Broad exposure range (2.5, 25, 250, 2500, and 25,000 µg/kg body wt. /day)
- Measurement of internal exposure (blood) in kinetic studies; measurement of background levels in study materials
- Concurrent positive control: ethinyl estradiol (0.05 and 0.5 µg/kg body wt. /day); negative controls included untreated animals and those gavaged with the vehicle only.
- Study length: core GLP- study examined animals at 1 and 2 years; animals from grantee studies at PND 1,15, 21, and 90 and at 6 mos. and 1 year.

CLARITY-BPA Study Design (continued)

- 50 animals per dose group, per sex, per age in core study; additional animals provided for grantee studies
- Potential sources of background BPA including diet, cages, bedding, drinking water, and water bottle stoppers were measured and monitored.
- Animals were fed a diet low in soy, alfalfa, and other phytoestrogens, and several potential dietary estrogens, and BPA levels in the chow were measured over the course of the study. The consideration of the base diet as a potential source of BPA contamination was another unique and important aspect of the core study.
- Assessment of low-dose BPA and ethinyl estradiol blood levels were determined.
- In summary, all aspects of the CLARITY-BPA study followed a shared, robust protocol for animal housing and feeding, compound handling and dosing, measurements and evaluation, and sharing and storing of samples.

CLARITY-BPA Grantees and areas of study

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Gail Prins	Prostate cancer	Prostate gene expression, and cancer development PND 21; 6, 12 and 24 months	1) prostate gene expression 2) prostate methylation 3) renewal of stem cells 4) assess PIN and cancer
Heather Patisaul/ Cheryl Rosenfeld	Learning and Behavior	Brain transcriptomics *birth* Behavioral *PND 21 and 90*	1) brain gene expression 2) behavioral assess. pnd 21 3) behavioral assess. pnd 90
Norbert Kaminski	Immune function	Spleens assessed *PND 90; 12 months*	1) spleen T & B cell subpopulations 2) response to stimulation 3) ER characterization 4) gene expression

CLARITY-BPA Grantees and areas of study

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Kim Boekelheide	Testis function/ sperm counts Continuous dosing only	Testis and epididymis *PND 90; 12 months*	1) histological and morphological assessment of testis 2) caudal sperm transcriptome 3) caudal sperm methylome
Ana Soto	Breast cancer	Breast development and cancer *PND 21 and 90; 6 months* *(whole mounts)*	1) breast morphology at pnd 21 as precursor of cancer 2) gene expression and DNA methylation at pnd 21 3) assess pre-neoplastic lesions and neoplastic lesions pnd 90 and 6 mo.
Shuk Mei Ho	Uterine cancer Continuous dosing only	Uterus histology and gene expression *6, 12, 24 months*	1) histological ID of uterine hyperplasia/adenocarcinoma 2) laser capture to assess methylome and transcriptome to identify early cancer genes

CLARITY-BPA Grantees and areas of study

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Nira Ben Jonathan	Obesity/adipose tissue	adipose tissue disposition and weight gain *PND 90; 6 and 12 months*	1) fat depots and selected adipokines, gene expression 2) serum hormones 3) adipose cell number & size 4) BPA in fat tissues
Fred vom Saal	Male urogenital abnormalities	Urogenital system analysis *Birth; 12 and 24 months*	1) 3-D reconstruction of urogenital system 2) examine animals for voiding and laser capture to assess gene expression in epithelium and stroma
Jodi Flaws	Ovarian function	Ovary Birth; pnd *21, 90; and 12 months*	1) follicle number 2) steroidogenic enzymes

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Tom Zoeller	Thyroid and brain anatomy	Thyroid and brain development *PND 15 and 21*	1) changes in brain gene expression and histology due to BPA impact on thyroid hormones
Nestor Gonzalez-Cadavid	Penile function	Penile erection mechanism *12 months*	1) erection capability, transcriptomic profile and stem cell analysis
Andrew Greenberg	Diabetes, blood glucose, pancreas	Blood glucose and pancreas assessment 12 months	1) assess blood glucose over time and beta cell mass and insulin content

Summary of Some Diseases Addressed by CLARITY- BPA

- Prostate
  - Cancer (Pin)
  - Urethral obstruction (BPH)
- Decreased sperm counts
- Penile dysfunction
- Cardiovascular
- Immune (sensitivity to infections, asthma)
- Transgenerational (3rd generation)
- *Low Dose effects*
- *Gene expression/ epigenetics*
- Breast cancer
- Uterine cancer
- Ovarian toxicity
  - Oocyte quality in IVF
- Obesity
- Diabetes
- Early puberty
- Brain effects
- Learning and memory
  - Anxiety/motivation
  - Social behavior
  - Sex differences

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