NTP Update:
BPA Studies and a Cooperative (UO1)
BPA Research Consortium

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NTP Board of Scientific Counselors Meeting
December 11, 2012
Relevant History

• August 2008 - FDA released draft assessment of BPA for use in food contact applications

• September 2008 - FDA Science Board reviewed draft assessment
  – Agreed with some parts of the assessment
  – Cited limitations in justification for exclusion of non-GLP studies
  – Recommended evaluation of studies considered adequate by the NTP-CERHR review (carried out in 2007), plus additional newer studies
  – Stated margins of safety defined by FDA may be inadequate

• FDA reported its review of studies supporting NTP's conclusions of “some concern” in August 2009
  – “The NTP has some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A”

Relevant History (continued)

- August 2009 - FDA released assessment of literature supporting effects on brain and behavior, prostate, mammary gland, and age at which females attain puberty (additional endpoints in draft NTP conclusions)
  - Extensive documentation of criteria for inclusion in safety assessment
  - Discussion of neurobehavioral and developmental findings in relation to adverse effects, identifying areas of uncertainty
  - Discussion of mammary gland developmental changes and possible relationship to cancer
  - Discussion of uncertainty of prostate and male urogenital tract findings with respect to longer term outcomes
  - Discussion of inconsistency of reports of effects on onset of puberty, need for careful replication
  - Discussion of epigenetic findings, glucose homeostasis, and pancreatic function, need for further development of literature
  - Maintained the 5 mg/kg/day NOAEL
Studies Underway and Completed

- By 2008, NIEHS had funded 39 investigator-initiated grants
- In 2009, NIEHS awarded 10 Grand Opportunity and 3 Challenge Grants under the American Recover and Reinvestment Act (ARRA) program
- Also in 2009, NIEHS created the BPA Grantee Consortium
  - Meet in-person yearly and by conference call once per month
  - Publications to date:
- Human oral clinical pharmacokinetic studies underway, dermal in design
- Exposures from dental materials and thermal receipts reported or underway
- Occupational exposure assessments with NIOSH in design phase
- NTP-supported rodent and monkey kinetic studies at NCTR reported
- NTP-supported 90-day perinatal exposure studies in the NCTR SD rat at NCTR (data in review)
U01 NIEHS Academic Grantees Consortium

- U01 is a cooperative agreement establishing a research consortium
- Each principal investigator serves on a steering committee
- Decisions about design, performance, and reporting are consensus
- Monitor progress, recommend redirection if needed
- Articles of Collaboration established detailing specific responsibilities
- Samples provided to investigators blinded, code broken after submission of data to NIEHS’ Chemical Effects in Biological Systems (CEBS) database
- Investigators may publish independently, but all data shared and available for integrated assessments
- Panel of academic advisors appointed
NTP-NCTR Studies

- Modified “guideline-compliant” studies (subchronic/chronic) to include endpoints not typically measured
- Direct dosing of neonates by gavage, 7 days per week; broad exposure range
- Measurement of internal exposure (blood) in kinetic studies; measurement of background levels in study materials
- Concurrent ethinyl estradiol (EE₂) control (2 dose levels)
- Subchronic study: Design presented at BPA Grantee Consortium and FDA Science Board meetings, Sept. 2009 with offer of tissue sharing; a few tissues requested
- Chronic study: Extensive preplanned sharing of tissues/animals with academic laboratories receiving extramural funding (UO1)
Chronic Study GLP Protocol

• Vehicle control, two EE₂ controls plus 5 BPA dose groups (2.5 - 25,000 μg/kg bw/day, 10-fold spacing)

• SD rat from NCTR colony; exposure starts at GD 6
  – Dams dosed by gavage until litters born
  – Pups dosed directly starting at PND 1

• One male and one female from 50 litters from each dose group and control for 2-year evaluation, continuous dosing

• One male and one female from 50 litters from each dose group and control for 2-year evaluation, stop dosing at PND 21

• One male and one female from 50 separate litters for interim (1 year) evaluation; one-half continuous dosing, one-half stop dose at PND 21

• Other pups go to funded NIEHS grantee studies
Chronic Study GLP Protocol Elements

• Core protocol for interim (1 year) and 2-year animals
  – Vaginal cytology starting at 4 months to evaluate onset of aberrant cycles
  – Clinical chemistry, sperm analysis, organ weights, and target organ histopathology on interim sacrifice animals
  – At 2 years, complete necropsy with selected target organ histopathology

• Subset of animals for behavior testing

• All other animals for NIEHS-funded grantee studies; tissues from the same animals shared when feasible
# Consortium Members and Areas of Study

<table>
<thead>
<tr>
<th>Name</th>
<th>Disease Focus</th>
<th>Endpoint</th>
<th>Aims Funded</th>
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</table>
| Gail Prins         | Prostate cancer                | Prostate gene expression and cancer development *(PND 21; 6, 12, and 24 months)* | • Prostate gene expression  
• Prostate methylation  
• Renewal of stem cells  
• Assess PIN and cancer  |
| Heather Patisaul   | Learning and behavior          | Brain transcriptomics *(Birth)*  
Behavior *(PND 21 and 90)* | • Brain gene expression  
• Behavioral assessment *(PND 21 and 90)*  |
| Norbert Kaminski   | Immune function                | Spleen assessed *(PND 90 and 12 months)*                                 | • Spleen T and B cells subpopulations  
• Response to stimulation  
• Estrogen receptor (ER) characterization  
• Gene expression  |
| Kim Boekelheide   | Testis function/sperm counts *(Continuous dosing only)* | Testis and epididymis *(PND 90 and 12 months)* | • Histological and morphological assessment of testis  
• Caudal sperm transcriptome  
• Caudal sperm methylome  |
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<tbody>
<tr>
<td>Ana Soto</td>
<td>Breast cancer</td>
<td>Breast development and cancer (PND 21 and 90; 6 months (whole mounts))</td>
<td>• Breast morphology as precursor of cancer ((PND\ 21))</td>
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<td>• Gene expression and DNA methylation ((PND\ 21))</td>
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<td>• Assess pre-neoplastic lesions and neoplastic lesions ((PND\ 90\ and\ 6\ months))</td>
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<td>Shuk Mei Ho</td>
<td>Uterine cancer (\text{Continuous dosing only})</td>
<td>Uterus histology and gene expression ((6, 12, \text{and}\ 24\ months))</td>
<td>• Histological identification of uterine hyperplasia/adenocarcinoma</td>
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<td>• Laser capture to assess methylome and transcriptome to identify early cancer genes</td>
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<td>Nira Ben</td>
<td>Obesity/adipose tissue</td>
<td>Adipose tissue disposition and weight gain ((PND\ 90;\ 6\ \text{and}\ 12\ months))</td>
<td>• Fat depots and selected adipokines, gene expression</td>
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<tr>
<td>Jonathan</td>
<td></td>
<td></td>
<td>• Serum hormones</td>
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<td>• Adipose cell number and size</td>
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<td></td>
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<td>• BPA in fat tissues</td>
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| Fred vom Saal         | Male urogenital abnormalities          | Urogenital system analysis *(Birth; 12 and 24 months)*                   | • 3D reconstruction of urogenital system  
• Examine animals for voiding and laser capture to assess gene expression in epithelium and stroma |
| Jodi Flaws            | Ovarian function                       | Ovary *(Birth, PND 21 and 90, and 12 months)*                           | • Follicle number  
• Steroidogenic enzymes                                                                          |
| Tom Zoeller           | Thyroid and brain anatomy              | Thyroid and brain development *(PND 15 and 21)*                         | • Changes in brain gene expression and histology due to BPA impact on thyroid hormones         |
| Nestor Gonzalez-Cadavid | Penile function                        | Penile erection mechanism *(12 months)*                                 | • Erection capability, transcriptomic profile, and stem cell analysis                         |
| Andrew Greenberg      | Diabetes, blood glucose, and pancreas  | Blood glucose and pancreas assessment *(12 months)*                     | • Assess blood glucose over time, beta cell mass, and insulin content                         |
Diseases Addressed in BPA Guideline Study*

- 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

Key
Blue = included NTP-NCTR study
Black = reported in the literature
Italics = included by multiple investigators within NTP-NCTR study
Acknowledgements

• NCTR
  – Barry Delclos
  – Paul Howard
  – Luisa Camacho
  – Dan Doerge
  – Sherry Lewis

• NIEHS
  – Jerry Heindel
  – Thad Schug
  – Nigel Walker
  – Retha Newbold (contractor)
Model Selection

• Rat, and often the SD rat, is the primary model used in preclinical reproductive and developmental data submitted to FDA and other regulatory agencies
  – Effects in low dose range for BPA have been reported in SD rats, including Charles River SD rats (both *in vivo* and *ex vivo* studies)
  – Multigenerational reproductive / chronic dietary studies conducted with NCTR SD rat (genistein, EE₂)
• Also notable that EFSA (2010 opinion), which upheld the current TDI of 50 µg/kg bw/day, cited studies in SD rat (CR) as low dose effects of concern requiring further study
Chronic Study Dose Selection

- 0, 2.5, 25, 250, 2,500, 25,000 μg BPA/kg bw/day

- Low dose range
  - No clear adverse effects in the subchronic study, but there were sporadic significant effects, and some significant effects in a parallel neurobehavioral pilot study at 2.5 and 25 μg BPA/kg bw/day
  - 2.5 to 10 μg BPA/kg bw/day identified as LOAELs or NOAELs in literature

- High dose range
  - High dose would provide clear adverse effect (~25,000-fold above estimated exposures)
  - Effects resembled EE₂ in subchronic study