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

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# 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"

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. NTP, NIEHS, NIH 08-5994 (2008)

# 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:

  http://www.niehs.nih.gov/health/topics/agents/sya-bpa/bpa-related/index.cfm
- 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 ($EE_2$) 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 $EE_2$ 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

| Name | Disease Focus | Endpoint | Aims Funded |
|---|---|---|---|
| Gail Prins | Prostate cancer | Prostate gene expression and cancer development *(PND 21; 6, 12, and 24 months)* | • Prostate gene expression<br>• Prostate methylation<br>• Renewal of stem cells<br>• Assess PIN and cancer |
| Heather Patisaul | Learning and behavior | Brain transcriptomics *(Birth)*<br>Behavior *(PND 21 and 90)* | • Brain gene expression<br>• Behavioral assessment (*PND 21 and 90)* |
| Norbert Kaminski | Immune function | Spleen assessed *(PND 90 and 12 months)* | • Spleen T and B cells subpopulations<br>• Response to stimulation<br>• Estrogen receptor (ER) characterization<br>• 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<br>• Caudal sperm transcriptome<br>• Caudal sperm methylome |

# Consortium Members and Areas of Study

| Name | Disease Focus | Endpoint | Aims Funded |
|---|---|---|---|
| Ana Soto | Breast cancer | Breast development and cancer *(PND 21 and 90; 6 months (whole mounts))* | • Breast morphology as precursor of cancer (*PND 21)*<br>• Gene expression and DNA methylation (*PND 21)*<br>• Assess pre-neoplastic lesions and neoplastic lesions (*PND 90 and 6 months)* |
| Shuk Mei Ho | Uterine cancer *Continuous dosing only* | Uterus histology and gene expression *(6, 12, and 24 months)* | • Histological identification of uterine hyperplasia/adenocarcinoma<br>• Laser capture to assess methylome and transcriptome to identify early cancer genes |
| Nira Ben Jonathan | Obesity/adipose tissue | Adipose tissue disposition and weight gain *(PND 90; 6 and 12 months)* | • Fat depots and selected adipokines, gene expression<br>• Serum hormones<br>• Adipose cell number and size<br>• BPA in fat tissues |

# Consortium Members and Areas of Study

| Name | Disease Focus | Endpoint | Aims Funded |
|---|---|---|---|
| Fred vom Saal | Male urogenital abnormalities | Urogenital system analysis *(Birth; 12 and 24 months)* | • 3D reconstruction of urogenital system<br>• 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<br>• 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 ($3^{rd}$ 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

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  - 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, $EE_2$)
- 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 $EE_2$ in subchronic study