NCTR/FDA Research on BPA: Integrating pharmacokinetics in animal models and humans, rat toxicology studies, human biomonitoring, and PBPK modeling into risk assessment

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Research support by Interagency Agreement between NTP/NIEHS and NCTR/FDA
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INTRODUCTION

- BPA is a high production volume chemical used for polycarbonate plastic and epoxy can liners
- Trace levels widespread throughout the environment (indoor and outdoor)
- Exposure is predominantly through the diet (>90%) since daily intake estimates from food consistently exceed aggregate excretion reconstructions from urinary biomonitoring
- Potential health effects controversial despite extensive (unprecedented) study
- FDA regulates safety of food contact materials and medical devices
NCTR/FDA STUDY GOALS (2009)

- Expand PK data from mice, rats, monkeys, and humans to create and validate PBPK models that will predict internal exposures to BPA in target tissues of adults, fetuses, and babies from food contact materials and medical devices.

- Conduct large-scale rat studies under strictly controlled conditions with a multiplicity of doses in the low dose region administered throughout all lifestages to create comprehensive toxicological evaluations of BPA.
OUTLINE

- Analytical Methodology
- Adult PK – rodents & primates
- Persistence – serum and tissues
- Neonatal PK – rodents & primates
- Pregnant/Fetal PK – rodents & primates
- Human PK
- Human urine and blood biomonitoring
- BPA 90-d toxicity evaluation in SD rats
- Possible use in risk assessment
Analytical Methodology

- LC/MS/MS (sensitivity and selectivity)
- $^{13}$C-labeled IS (isotope dilution)
- Method validation and QC
- Native BPA can contaminate any step in sample handling chain (plastics, dust, solvents, …)
- Same problem for other ubiquitous environmental trace contaminants – phthalate diesters, dioxins, …
- Isotopically labeled d6-BPA dosing material
- LOD 0.004-0.2 nM serum; 0.4 pmol/g tissue
- Common experimental design – 100 $\mu$g/kg bw oral and IV dosing for inter-species comparisons
- Aglycone (parent compound = active) and Phase II conjugates (inactive)
Oral Bioavailability of BPA in Adults Is Low (<1% in **Monkey** and Mouse; <3% in Rat)
BPA is Extensively Conjugated in Adult Monkeys After Oral and IV Adminstration
BPA Is not Persistent in Serum or Adipose Tissue from Adult CD-1 Mice (IV)
$C_{\text{max}}$ for Oral BPA Decreases with Post-natal Age in Rodents, but not Monkeys
Phase II Metabolism Increases with Post-natal Age in Neonatal Rodents, but not Neonatal Monkeys
Fetal Rat Exposures to BPA are Similar to Other Maternal Tissues and Decrease with Gestational Age

![Graph showing Fetus/Dam Partition Coefficient for GD12, GD16, GD20, and Uterus, with values decreasing with gestational age.](chart.png)
Rat Phase II Metabolism of BPA Increases Throughout Perinatal Period
Simultaneous Maternal/Fetal BPA PK in Rhesus Monkeys

- IV bolus administration of d6-BPA to mother @100 μg/kg bw
- GD 121-139 out of 164 d term
- Simultaneous blood sampling from maternal and fetal circulation
- Measurements of d6-BPA in maternal and fetal serum, amniotic fluid, and placenta
Fetal Monkey Exposures to BPA are Lower than Maternal Levels (AUC 45%)
Fetal Aglycone BPA is Eliminated Despite Persistent Levels of Conjugates
Human Serum Pharmacokinetics of d6-BPA 
Confirm and Extend Previous Studies

![Graph showing the pharmacokinetics of d6-BPA in human serum. The graph plots time (h) on the x-axis and [BPA] (nM) on the y-axis. Two lines are shown: one for Total BPA and another for Aglycone BPA. The data points are marked with error bars.](image)
CONCLUSIONS – Methodology

• Analytical methodology critical – sensitivity/specificity, validation, blanks (collection, storage, analysis), QC
• Contamination by ubiquitous native BPA (aglycone) haunts measurements in blood and interpretation of human exposures
• Urine less problematic than blood-based measurements (sterile collection, lipid and protein content, volume)
• Urine concentrations of total BPA >> blood (>40-fold)
• Urine-based biomonitoring of total BPA chosen for national exposure estimates of aggregate BPA exposure (NHANES-CDC)
CONCLUSIONS – Adult PK

- BPA oral bioavailability is low in adults of all species – rapid sequential metabolism in the GI tract and liver
- Systemic Phase II metabolism substantial even after parenteral administration
- Quantitatively excreted – 70% bile/30% urine with EHR in rodents; ~100% urine in primates
- Non-persistent, even in adipose tissue
CONCLUSIONS – Perinatal PK

- Phase II metabolism is deficient at birth in rodents and produces ~10-fold higher internal exposures vs. primates.
- Neonatal rodents are predicted to be more sensitive than primates based on internal exposure.
- Fetal exposures are ≤ maternal levels and fetal Phase II metabolism increases throughout gestation.
- Placenta - metabolically active tissue (Phase II); no evidence for deconjugation in vivo.
- Amniotic fluid levels < maternal serum for both aglycone and conjugates.
- Evidence for absence of selective fetal accumulation/deconjugation of aglycone BPA.
- Maternal, fetal (placental) Phase II metabolism reduce fetal exposures to aglycone BPA.
CONCLUSIONS - Human Oral PK

- Consistent with previous controlled oral exposure studies in humans and animals
- Low bioavailability; quantitative urinary excretion
- Paired urine and blood analysis shows total BPA is consistently >40-fold higher in urine than serum (aglycone < 1% in both)
- Urinary biomonitoring approach validated
- Data provide benchmarks for identifying blood contamination by aglycone at >1% (oral) and >15% (IV)
- Allometric scaling of systemic clearance values for BPA shows consistent dependence on physiological processes between rodent and primate species
- PBPK modeling approaches validated
- Dosing inputs: current dietary intake estimates in adults: 0.1-0.3 μg/kg bw (mean-90th) vs. aggregate daily intake 0.04-0.12 (median-95th, NHANES)
PBPK simulations of BPA ingestion in adults 3 meals, 0.1 (mean) and 0.3 (90\textsuperscript{th}) \(\mu\)g/kg bw/d
PBPK simulations of BPA ingestion in infants
6 meals, 0.3 (mean) and 0.6 (90th) µg/kg bw/d

![Graph showing BPA and BPA-G concentrations over time](image)
BPA Toxicology Studies: Research-Scale vs. Guideline-Compliant

- Common criticisms of many research-scale studies reporting low dose effects of BPA include limited dose range, limited animal numbers, inappropriate statistics, lack of control/reporting of background BPA and dietary soy phytoestrogens, lack of internal dosimetry measurements, inappropriate routes of administration
  - Commonly not examining endpoints considered as evidence for adverse effect in risk assessments
- Guideline-compliant studies of dietary BPA in rats and mice (e.g. Tyl et al., 2002, 2008) do not suffer from many of these deficiencies
  - No internal dosimetry or diet measurements
  - Significant attenuation of dose administered through milk at critical developmental period for pups
  - Human exposure of concern involves direct consumption by infants
Rationale for Studies Conducted Under FDA- NCTR/NIEHS-NTP Interagency Agreement

- Rat model – NCTR Sprague-Dawley (SD)
  - Appropriate animal model for testing has been a subject of controversy
  - Low dose BPA effects reported in literature in SD rats from multiple sources
  - Previous dietary administration studies had shown sensitivity of NCTR SD rat to dietary EE$_2$ and genistein in multigenerational reproductive and chronic studies under similar conditions (historical controls)
  - Extensive PK work on BPA completed in this animal
  - SD rat is commonly used in reproductive toxicity testing
BPA Subchronic (90-d) Study Design Elements (Delclos et al., 2014; Churchwell et al., 2014)

- **Treatment groups**
  - 7 Equally spaced BPA doses in “low” dose range (2.5, 8, 25, 80, 260, 840, 2,700 μg/kg bw/d)
  - 2 high BPA doses (100,000 and 300,000 μg/kg bw/d)
  - Two doses of reference estrogen, EE2 (0.5 and 5.0 μg/kg bw/d)
    - Doses selected based on previous dietary study
  - Vehicle (0.3 % carboxymethylcellulose) and naïve controls
  - n ~ 20 litters per dose group (18 – 23)

- **Gavage dosing of dams from GD 6 until parturition**
  - Direct oral gavage of pups from PND 1
  - Extensive evaluation of internal dosimetry (controls, BPA, and EE2; endogenous steroid hormones)
  - Combined genistein and daidzein < 2 ppm in diet
  - BPA cutoff 5 ppb in diet
  - Drinking water, cage materials, bedding, vehicle also analyzed
BPA 90-d Study Design Elements

• Measured serum levels of test compounds at $\sim C_{\text{max}}$ [30 – 60 min after dosing] at PND 4, 21, and 80
• Focus on histopathology and clinical chemistry for reproductive endpoints, but included additional endpoints to cover some reported BPA effects outside reproductive tract (e.g., insulin, glucose, adipose tissue, heart)
• Primary interest was BPA $\leq 2,700 \ \mu g/kg \ \text{bw/d}$
• Statistical comparisons to vehicle conducted in predefined subgroups
• Pathology Quality Assessment Review and Pathology Working Group assessment of slides and study pathologists’ findings conducted in 2012
General Summary of 90-d Findings

- **EE2**
  - Low dose: multiple effects in females, generally less severe than high dose; males not affected except for increased hyperplasia of mammary gland
  - High dose: both females and males affected, with females showing greater response

- **BPA**
  - Low doses (2.5 – 2,700 μg/kg bw/d): no effects distinguishable from background
  - 100,000 μg/kg bw/d decreased gestational weight gain
  - 300,000 μg/kg bw/d: multiple adverse effects in both sexes, some similarities to EE2 (ovarian pathology, aberrant cycles in females), but other toxicity evident (e.g. reduced preweaning survival, reduced body weights in both sexes)
ER$\alpha$ Binding-Equivalent Dosimetry in 90-d BPA Toxicity Study
Risk Assessment – Combining Human Exposure and Animal Toxicity

- Toxicology testing for regulatory risk assessment specifically includes effects on endocrine-regulated processes as well as organ-specific toxicities, reproductive toxicity, and carcinogenicity
- Well-defined perinatal exposures
- Clear link to adversity critical (histopathology)
- BPA acts through estrogenic mechanisms only at the highest dose - 300,000 μg/kg bw/d based on overlap with effects in female rats dosed throughout life stages (GD 6-PND 90) with those from reference estrogen and ERα occupancy dosimetry
- No biologically significant effects from BPA at doses of 2.5-2,700 μg/kg bw/d (NOAEL >2,700 μg/kg bw/d)
- U.S. dietary intake (infants) : 0.3-0.6 μg/kg bw/d
- Margin of External Exposure (infants) >9,000 (mean) and >5,000 (90th)
Convergence of Theory, Data, and Simulation: Human internal exposure (serum) to BPA is in the pM range (Teeguarden et al., 2013)