Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA): Core Study

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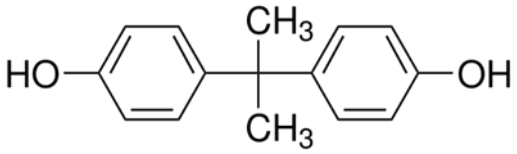
Any opinions conveyed in this presentation are those of the speaker and do not necessarily reflect those of the FDA or NTP

Outline

- Background on BPA research conducted at NCTR
- Conception and implementation of CLARITY-BPA
  - Selection of academic projects
  - Planning of core study design
  - Compromises
- Study conduct and reporting

Bisphenol A (BPA)

- Chemical widely used to make polycarbonate plastics & epoxy resins (used in can linings)



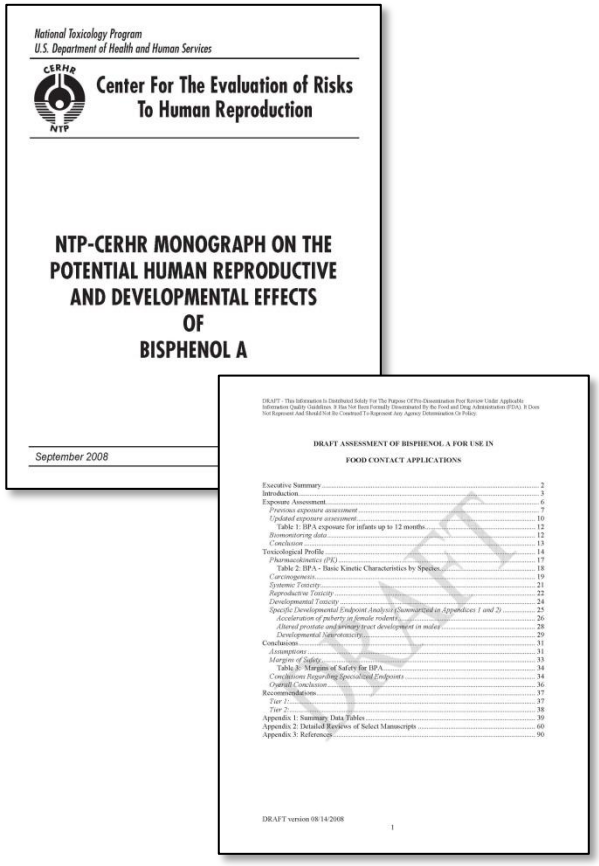
- Widespread low exposure (<0.5 µg/kg bw/day) from migration of small amounts into foods from food contact materials (indirect food additive)

Considerable Debate Over Risk Posed by “Low Level” BPA Exposure

- Guideline studies show no effects of concern at “low doses”
- Many academic “investigative” studies report that BPA induces a variety of effects in a variety of model systems at relatively low exposures
  - Non-monotonic dose responses reported, such that effects observed at high doses may not predict effects at lower doses
- Weak estrogen agonist, but multiple other mechanisms of action proposed

FDA/NCTR Research Program on BPA

- Designed to specifically address data gaps identified in the:
  - US National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP CERHR) 2008 review
  - US FDA Center for Food Safety and Nutrition (CFSAN) 2008 Draft Assessment of BPA for Use In Food Contact Applications
  - FDA Science Board review of 2008 Draft Assessment



FDA Science Board Key Recommendations

- Rodent study should be considered….designed to:
  - Meet criteria for acceptance established by the FDA or reasonable criteria applied by the scientific community for study evaluation that FDA should adopt
  - Address the endocrine mechanism-based concerns of the scientific community
  - Use endpoints and models validated for the study of endocrine-mediated developmental processes

- Develop PBPK models for model species and humans
  - Enable comparisons of dose to be made across species in a rational, quantitative manner

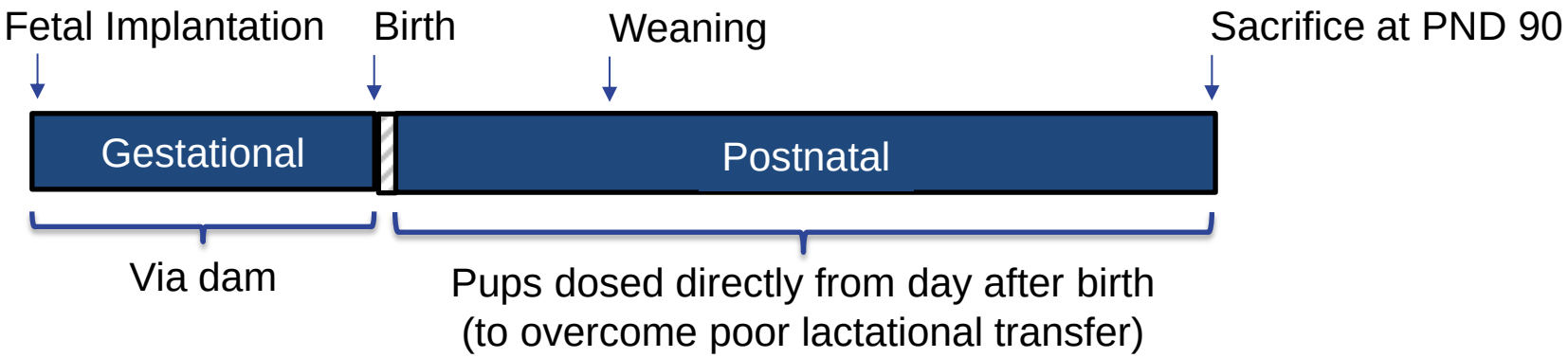
https://www.fda.gov/ohrms/dockets/ac/08/briefing/2008-4386b1-05.pdf

FDA/NCTR Research Program on BPA

- Pharmacokinetic studies
  - Multiple species (mouse, rat, nonhuman primate)
  - Multiple routes (oral, subcutaneous, intravenous)
  - Multiple life stages (fetal, neonatal, juvenile, adult, pregnant)
  - Human, oral dosing
  - All studies used deuterated BPA to avoid confounding by environmental BPA, sensitive and specific liquid chromatography tandem mass spectrometry method
- Physiologically based pharmacokinetic models
  - Developed models for rat and human
  - Allow extrapolation across routes, doses, and species
- Toxicology studies

BPA 90-Day Subchronic Toxicology Study

- NCTR Sprague-Dawley rat (CD23/NctrBR)
  - Used in previous multigenerational/chronic dietary administration studies of genistein, nonylphenol, ethinyl estradiol (EE_2)
  - Used for comprehensive evaluations of BPA pharmacokinetics across life stages
- Special housing and diet to minimize exposure to BPA and xenoestrogens (*e.g.*, genistein)
  - BPA levels monitored in cages, bedding, water bottles, and feed
    - Only feed showed BPA levels above background, < 5 ppb
- Oral dosing by daily gavage, 7 days/week



BPA 90-Day Subchronic Toxicology Study

- Exposure groups:
  - Naïve control (not dosed by gavage)
  - Vehicle control
  - “Low” BPA: 2.5, 8, 25, 80, 260, 840, and 2,700 µg/kg body weight/day
  - “High” BPA: 100,000 and 300,000 µg/kg body weight/day
  - Reference estrogen controls (EE_2): 0.5 and 5 µg/kg body weight/day
- Body/organ weight, sexual maturation, sperm analysis, vaginal cytology, clinical pathology, histopathology
- Internal dosimetry across life stages
- Gene expression and DNA methylation

BPA 90-Day Subchronic Study – Main Findings

- No differences between naïve and vehicle controls
- EE_2 induced dose-dependent clear adverse effects
  - Females more sensitive than males
- "High" BPA induced clear, dose-dependent adverse effects
  - Overlap with reference estrogen control
  - Females more sensitive than males
- "Low" BPA induced sporadic statistical effects of questionable biological significance

From Delclos *et al*. (2014); Churchwell *et al*. (2014); Camacho *et al*. (2015)

CLARITY-BPA

- Proposed by NIEHS Division of Extramural Research and Training (DERT) and NTP while the 90-day subchronic study was underway
- Novel research collaboration model to bridge investigative and applied-regulatory science research
- Consortium of NIEHS-funded academic researchers with federal scientists and regulators (FDA/NCTR, FDA/CFSAN, NIEHS/NTP, and NIEHS/DERT)
- Two key components:
  - NCTR core chronic study
  - Academic grantees studies

Schug *et al*. (2013); Heindel *et al*. (2015)

2010: NIEHS Funding Opportunity Announcement

- Develop a consortium of researchers to work with the NCTR and NTP in final design of chronic gavage toxicity study of BPA in NCTR-SD rats following developmental and direct rather than lactational exposure of pups
- Proposals solicited for hypothesis-driven mechanistic studies focusing on disease/dysfunction endpoints that could be added to the chronic study design
- Proposals reviewed and selected by an NIEHS peer review committee in 2011
- FDA (CFSAN, NCTR) did not have input into study selection
- NCTR reviewed selected proposals only for feasibility
  - No studies selected by the NIEHS peer review were refused, although modifications were necessary

Goals of CLARITY-BPA

- Evaluate chronic exposure to BPA over a broad dose range using traditional and non-traditional endpoints
- Determine if non-traditional endpoints reveal toxicity not detected by traditional guideline study endpoints
- Determine if non-traditional endpoints provide mechanistic support for observations made in the guideline study
- Dose-response assessment
  - Cover wide range of BPA doses with reported effects in the scientific literature

CLARITY-BPA Core Study Protocol Development

- A proposed protocol was initially developed in 2011 through the usual process used for studies conducted under the interagency agreement between NIEHS/NTP and FDA/NCTR
  - Proposal presented to Toxicology Study Selection and Review Committee (TSSRC) consisting of NIEHS, FDA product centers, and NCTR representatives in November 2011
  - Same animal model and dosing regimen as used in NCTR 90-day subchronic BPA study

CLARITY-BPA Core Study Protocol Development: Original Proposal

- 0, 2.5, 25, 250, 2500, 25000, and 250000 µg BPA/kg bw/day
  - Based on results of NCTR 90-day subchronic study

- Include one set of animals dosed continuously until termination at 1 or 2 years, a second set dosed only until weaning

- Received tentative approval of the TSSRC, pending input of academic grantees

Subsequent Modifications of Core Protocol

- The proposed protocol was presented to the CLARITY-BPA Consortium at a meeting in March 2012
- It was agreed that the proposed high dose of 250,000 µg BPA/kg bw/day was out of the range of concern and should be dropped
  - One investigator requested that a set of animals be dosed with this high level based on the results of the 90-day subchronic study
- The academic investigators argued for the inclusion of two dose levels of EE_2 to demonstrate the sensitivity of the system under study conditions
  - The dose levels of EE_2 selected were informed by the results of the 90-day subchronic study
  - Due to resource constraints, specifically space, an EE_2 stop dose arm could not be included in the chronic study, although it was included for academic investigators who requested it
  - The number of animals used in the EE_2 groups in the 2 year study were reduced for the core protocol due to space constraints

Compromises Required for Grantee Studies

- As mentioned previously, although no study selected by the NIEHS review process was refused, some proposals did need modification due to the size and resource (animal availability, space, technician support, etc.) requirements

- In most cases, grantee animal sets included 10 animals per sex per study arm per time point

- Several specific requests for additional manipulations could not be accommodated, examples:
  - A request to place a subset of animals on high fat diets and monitor body composition by MRI could not be accommodated
  - Sufficient animals were not available to provide the requested number of additional animals with testosterone and estradiol implants
  - Euthanasia methods could not be tailored for specific endpoints

- Final protocol was agreed to by all Consortium members

F_1 Animal Allocation at Weaning: Core Study

Sacrifice Time	Dosing Arm	Dose Group	Animals/Sex/Dose Group
1-year*	Continuous	Vehicle, BPA, EE_2	20-26
(interim)	Stop	Vehicle, BPA	19-22
2-years (terminal)	Continuous	Vehicle, BPA	46-50
		EE_2	26
	Stop	Vehicle, BPA	46-50

*Original target for 1-year groups was 26/sex/dose group, but this was reduced to afford animals to CLARITY-BPA academic studies

CLARITY-BPA Studies

Core animal study (NCTR)

Siblings

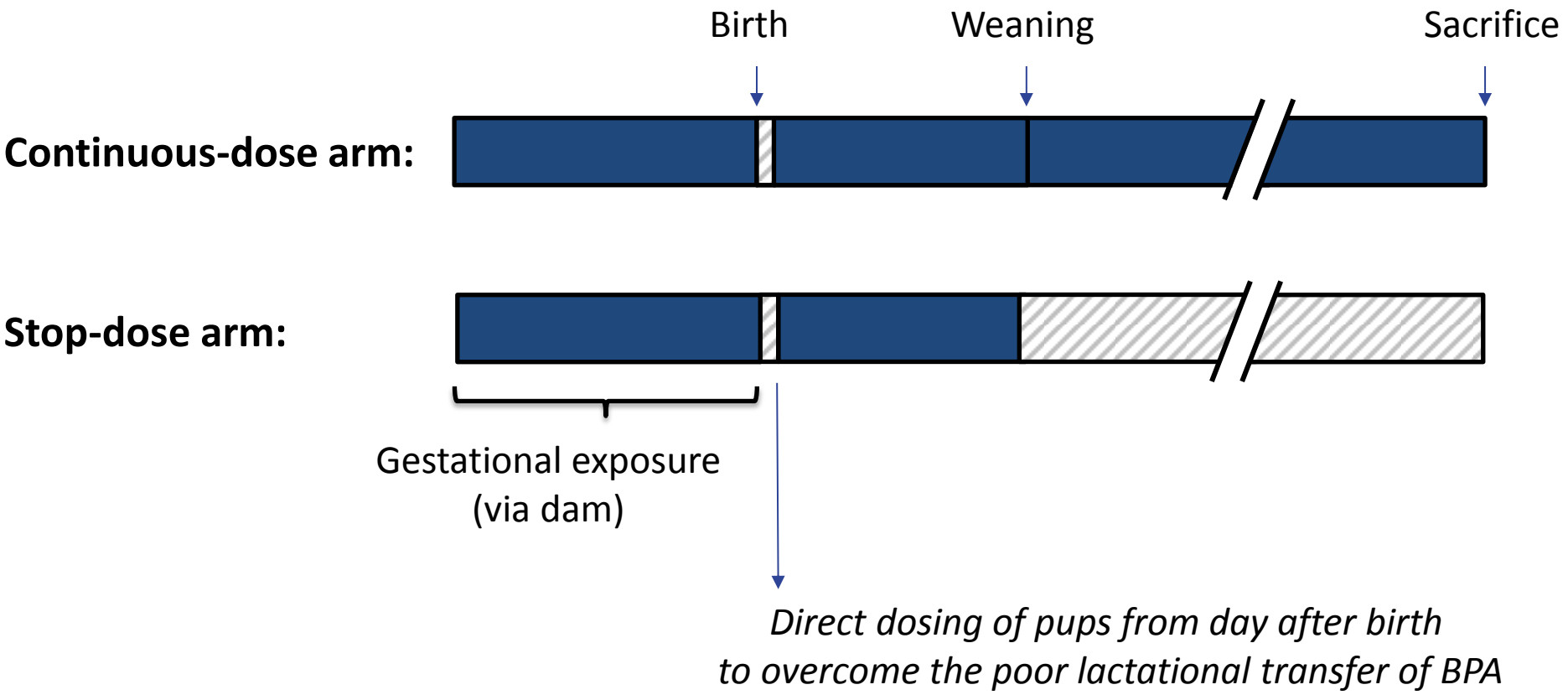
Core chronic study (NCTR)

- Modified guideline-compliant study
- 1 year necropsy (clinical chemistry, hematology, sperm parameters, organ weights, and histopathology)
- 2 year necropsy (histopathology)

Grantee studies

- Molecular, structural, and functional endpoints reported in association with BPA exposure in animal models or epidemiological studies
- Animals removed on postnatal days 1, 15, 21, and 90; 6 and 12 months (depending on study)
- Endpoints not typically assessed in guideline studies

Dosing Regimen



Study Elements Common to Core and Grantee Studies: Dose Levels

- Vehicle control: 0.3% aqueous carboxymethylcellulose
  - To ensure homogeneity of dosing suspensions (higher doses)

- Five BPA groups: 2.5, 25, 250, 2,500, and 25,000 µg/kg bw/day
  - Cover wide range of doses over which effects have been reported in the literature; typically chronic guideline studies use 3 doses over 4 – 10-fold dose range
  - Lowest dose set to be ≥10-fold higher than background dietary intake of BPA
  - Highest dose set to afford sufficient margin of exposure to current estimated human exposure to BPA; not based on “minimally toxic dose” from previous NCTR 90-day BPA study

- Two EE_2 groups: 0.05 and 0.5 µg/kg bw/day
  - Low dose was 10-fold lower than doses used in the previous NCTR 90-day subchronic study

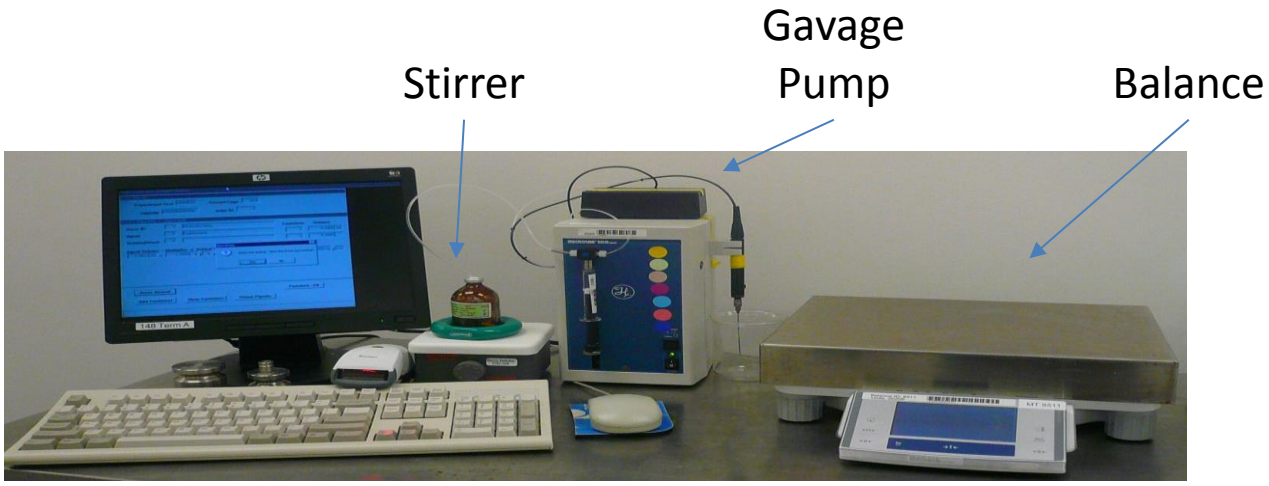
- Some grantees chose not to use both dosing arms or all dose levels

Study Elements Common to Core and Grantee Studies

- Doses
  - Identity and purity (>99%) of test articles certified
  - Dosing formulations certified to be within ± 10% of target dose and used within window of stability
- Animal housing
  - Polysulfone cages
  - Glass water bottles
  - Millipore-filtered water provided *ad libitum*
  - Hardwood chip bedding (AlphaDri cellulose bedding, when necessary)
  - All housing materials were monitored for BPA and certified not to contain BPA above analytical background levels
- Low phytoestrogen diet
  - BPA in diet < 5 ppb (~ 0.25 µg/kg bw/day ingested at this level); other contaminants monitored and reported

Study Elements Common to Core and Grantee Studies: Dose Administration

- Oral gavage, 7 days a week
- Automatically dispensed by Hamilton Microlab 500 pumps
  - Certified to deliver within ± 10% of target dose
  - Four dosing stations per animal room
    - Pump # 1: Vehicle
    - Pump # 2: BPA (2.5, 25, and 250 µg/kg bw/day)
    - Pump # 3: BPA (2,500 and 25,000 µg/kg bw/day)
    - Pump # 4: EE_2



Background Exposure to BPA

- Known: via feed (~0.03-0.2 µg BPA/kg bw/day)
- Presumed: via unknown source during the co-housing of animals with grantee animals being dosed with 250,000 µg BPA/kg bw/day (Load 0)

Animal Cohort	Highest BPA Dose in Room (µg/kg bw/day)	BPA-Glucuronide Detected in Vehicle Serum?
NCTR 90-day subchronic study	300,000	**Yes** (~2.5 BPA µg/kg bw/day)
CLARITY-BPA studies while co-housed with Load 0	250,000	**Not determined** (no serum available)
CLARITY-BPA studies not co-housed with Load 0	25,000 (10-fold lower)	**No**

- Analysis of BPA-CLARITY core study data included a sensitivity analysis that excluded all data from animals co-housed with animals dosed with 250,000 µg BPA/kg bw/day. There was no discernible impact.

Core Study Histopathology

- Performed by the Study Pathologist
- Evaluated by an independent quality assessment (QA) group
  - Pathology data review conducted by the QA pathologists
  - 100% review of slides from ovary, uterus, vagina, testes, epididymis, prostate, seminal vesicle, pituitary (♀,♂), and mammary gland (♀,♂)
- Reviewed by a Pathology Working Group (PWG), who evaluated:
  - Potentially treatment-related lesions
  - Disagreements in diagnosis between the Study Pathologist and the QA pathologists

Core Study Statistical Analyses

- Data analyzed within sex, dosing arm, and sacrifice time
- Litter was the unit of analysis
- BPA and EE_2 dose groups were compared to the vehicle control in separate analysis
- For non-histopathology, tests were two-sided and corrected for multiple comparisons
  - Exception: abnormal estrous cycle trend was one-sided
- Histopathology data analyses were one-sided, not corrected for multiple comparisons
- For all tests, $p<0.05$ set as significance level
- In final report, histopathology lesions were selected for discussion based on the low stringency statistical results and evaluated for biological plausibility

CLARITY-BPA Core Study Reporting

- NTP Draft Research Report and all data made available to the public in February 2018
- Peer-review meeting held at NIEHS in April 2018
- Final NTP Research Report included revisions based on comments received during the review process

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