

# **American Chemistry Council Polycarbonate / BPA Global Group**

## **Bisphenol A**

**Weight of Evidence Fails to Support  
Listing Under Proposition 65**

**DART IC Meeting**

**July 15, 2009**

- ***Executive Summary and Standard for Listing Under Proposition 65***

- Stanley W. Landfair  
*McKenna Long & Aldridge LLP*

- ***Review the Data***
- ***Apply the Proposition 65 Standard***

# Overview

- ***BPA Metabolism and Pharmacokinetics***
  - Steven G. Hentges, Ph.D.  
*American Chemistry Council*
- ***Evaluation of “Conventional” Animal Studies on BPA***
  - Rochelle W. Tyl, Ph.D., DABT  
*RTI International*
- ***Scientific Evaluation of BPA For Purposes of Proposition 65***
  - F. Jay Murray, Ph.D.  
*Murray & Associates*

# Standard for Listing Under Proposition 65

## ***Statute:***

“A chemical is known ... to cause ... reproductive toxicity ... if in the opinion of the state’s qualified experts it has been *clearly shown* through *scientifically valid testing* according to *generally accepted principles* to cause ... reproductive toxicity.”

# Standard for Listing Under Proposition 65 *(cont'd)*

## ***Duty of DARTIC:***

“Render an opinion ... as to whether specific chemicals have been *clearly shown*, through *scientifically valid testing according to generally accepted principles*, to cause reproductive toxicity.”

Cal. Code Regs., tit. 27, § 25305(b)(1)

# Guidance Criteria for Listing Chemicals as “Known to Cause Reproductive Toxicity”

## *General Principles:*

- “In evaluating the sufficiency of data, a ***weight of evidence*** approach shall be used to evaluate the body of information available for a given chemical.” Guidance Criteria at 1.D.
- “In determining whether a chemical is to be ... listed as known ... to cause reproductive toxicity, the ***biological plausibility*** of the association between the adverse reproductive effects observed and the chemical in question should be considered. Confidence is increased when ... a sound scientific basis exists for the observed adverse effects and the known characteristics of the particular chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical.” Guidance Criteria at 4.B.

# Guidance Criteria: Human Studies

***“Sufficient evidence in humans,” in the case of epidemiology studies, means studies that:***

- provide ***convincing evidence*** to support a ***causal relationship*** between exposure to the chemical in question and the ... effect in question.
- This requires ***accurate exposure*** and toxicity endpoint classification and proper control of ***confounding factors, bias***, and endpoint modifiers.

Guidance Criteria at 3.A.(1)

# Guidance Criteria: Animal Studies

***Whether animal studies are “sufficient evidence” to support extrapolation to humans, in most cases, is based on the following:***

- The ***experimental design*** and presence of appropriate controls
- The exposure, in terms of ***route of administration***, is relevant to expected human exposures
- The number of dose levels, so that the presence of a ***dose-response relationship*** can be evaluated
- Consideration of ***maternal and systemic toxicity***

Guidance Criteria at 3.C.(1)-(4)

# Standard for Listing under Proposition 65 *(cont'd)*

## ***Developmental Toxicity:***

- Proposition 65 regulates developmental effects caused by pre-natal exposures but not post-natal exposures.

(OEHHA General Counsel William Soo Hoo. DART Committee meeting. 1996.)

# Bisphenol A Metabolism and Pharmacokinetics

Implications for Proposition 65

Hazard Assessment

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DART Identification Committee Meeting

July 15, 2009

# Overview

- Metabolism and pharmacokinetics of BPA are well studied
  - Oral and parenteral (non-oral) routes of administration
  - Humans, non-human primates, rodents
- In humans, BPA is efficiently converted to non-estrogenic metabolites and rapidly excreted after oral exposure
- Significant inter-species differences between rodents and humans
- Route-dependent differences observed in animal studies

Metabolism and Pharmacokinetic Data Are  
Crucial for BPA Hazard Assessment

# Key Inter-Species Pharmacokinetic Differences

- In humans, extensive first-pass metabolism after oral exposure dramatically limits systemic BPA dose
  - Primarily BPA-glucuronide, some BPA-sulfate; both non-estrogenic
  - Half-life of metabolites ~4-6 hours; entirely excreted in urine; no bioaccumulation
  - Extensive metabolism also confirmed in premature infants
  - Hepatic glucuronidation capacity higher in humans vs. rodents
- In rodents, biliary excretion is primary elimination pathway
  - Enterohepatic circulation results in a significantly longer half-life and greater systemic bioavailability in rodents

Extrapolation of Any Effects in Rodent Studies  
to Humans is Tenuous

# Route-Dependent Pharmacokinetic Differences

- Large number of toxicity and mechanistic studies on BPA
  - Routes of exposure include oral and various non-oral routes
- Studies with oral route of exposure are most relevant for hazard assessment
  - Human exposure to BPA is oral through dietary sources
  - Extensive first-pass conversion to non-estrogenic metabolites
- Non-oral routes of exposure result exaggerates bioavailability; limited relevance for hazard assessment
  - No first-pass metabolism; parent BPA directly enters systemic circulation
  - Additional metabolites can also be produced

**Studies with Non-Oral Routes of Exposure  
are of Limited Utility and Relevance to Humans**

# Relevance of Weak Estrogenicity

- Weak estrogenicity of BPA demonstrated *in vitro*
  - Estrogenic potency generally 4-5 orders of magnitude below estradiol
  - Also lower potency than phytoestrogens common in diet
- BPA quickly converted to non-estrogenic metabolites after oral exposure
  - Human exposure to BPA is oral through dietary sources
- Biological plausibility of reproductive or developmental effects in humans through estrogenic mechanism is limited

Weak Estrogenicity of BPA is Not Relevant to Humans

# Pharmacokinetics and Epidemiology Studies

- Limited epidemiology data currently exist for BPA
  - Most current studies based on cross-sectional design
  - Biological samples for BPA measurement and health information collected at same time
- Humans rapidly eliminate BPA after oral exposure
  - Daily intake of BPA varies with diet, not likely to be constant over long periods of time
  - Measurement of BPA in spot urine or blood samples cannot reliably predict exposure in past or future

**Cross-Sectional Studies Cannot Determine Exposure  
During Etiologically Relevant Period**

BPA Proposition 65 Review  
Oakland, CA  
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*Reproductive and Developmental Hazard Assessment for Bisphenol A*

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# Tyl et al. Studies with BPA and E2 at RTI

- 1-Generation Study in Mice – BPA (abbreviated RACB study)
- 3-Generation Study in Rats - BPA
- 1-Generation Study in Mice – Estradiol (E2)
- 2-Generation Study in Mice – E2
- 2-Generation Study in Mice – BPA + E2 Positive Control

# 1-Generation BPA Study in Mice

- **Purpose:** to determine the F0 parental systemic toxicity of BPA at the high doses showing reproductive/developmental toxicity in the NTP RACB Continuous Breeding Study
  - NTP RACB study design does not evaluate F0 parental animals (i.e., maternal toxicity)
- **Design:** BPA dietary concentrations: 0, 5000, 10,000 ppm (approx. 800 and 1600 mg/kg/day)
- **Results:** parental female systemic toxicity at both doses
  - reduced body weights and feed consumption, increased liver and kidney weights; altered histopathology of liver (hepatocyte hypertrophy) and kidneys (renal tubule epithelial degeneration)

# 1-Generation BPA Study in Mice (Con't)

- **Results**

- 10,000 ppm only: As expected, reduced litter size (slightly reduced total and live pups/litter);
- No other significant effects on reproductive or developmental parameters

- **Conclusions**

- Effects on reproductive parameters only in the presence of maternal toxicity
- Reproductive effects in NTP RACB Continuous Breeding Study likely result from maternal toxicity

# 3-Generation Study with BPA in Rats - Strengths

Route of Administration:	Oral dietary (most relevant for human exposure)
Number of Dose Levels:	Six (0.001 to 500 mg/kg bw/day)
Number of Animals:	30/sex/dose group/generation
Endpoints Examined:	Multiple
Evaluated for Systemic, Reproductive, and Developmental Toxicity:	Males and females

# 3-Generation Study with BPA in Rats - Results

- Adult systemic toxicity at 50 and 500 mg/kg/day: reduced body weights and body weight gains, reduced absolute and increased relative weanling and adult organ weights, and female renal and hepatic toxicity at 500 mg/kg/day.
- Reproductive/developmental toxicity only at 500 mg/kg/day, which exceeded the MTD: decreased ovarian weights and total and live pups per litter, delays in acquisition of F1 male and female puberty not driven by estrogen activity but secondary to systemic toxicity in offspring; no effects on adult reproductive structures or functions.
- No BPA effects at low doses (0.001-5 mg/kg/day).
- No evidence for non-monotonic dose-response curves.
- BPA not considered a selective reproductive or developmental toxicant in rats.
- Reported to the FDA, EPA, EU, and OECD, at their request. Our results were confirmed at the NTP Endocrine Disruptors Low-Dose Peer Review in 2001, and our study was published in *Toxicological Sciences* in 2002.

# 2-Generation Study with BPA in Mice - Background

Request from the European Union (EU) for a BPA reproductive toxicity study in a second species (mouse) for the EU BPA Risk Assessment Committee Review

In preparation for a dietary BPA multigeneration study in mice with an E2 positive control group, we:

- Performed a one-generation dietary E2 study in CD-1 mice to determine:
  - range of dietary concentrations (ppm) and doses (mg/kg/day) within which the parental mouse females were able to get pregnant and carry live litters to term and beyond (to compare with the E2 data in CD/SD rats)
  - appropriate and sensitive endpoints to a potent endogenous estrogen and begin to collect negative (control) and positive (E2 exposed) data in the mouse

(published in *Reproductive Toxicology*, 2008)

## 2-Generation Study with BPA in Mice – Background (con't)

We also:

- Performed a subsequent two-generation dietary E2 study in CD-1 mice to:
  - Identify the appropriate dietary concentration (0.5 ppm) and dose (~0.080 mg/kg/day) to be used in the BPA study as a positive control
  - Confirm the relevant endpoints and appropriate responses to dietary E2 over two adult (F0 and F1) and two offspring generations (F1 and F2)

(published in *Toxicological Sciences*, 2008)

# Main Endpoints Affected by Dietary E2

- Prolonged gestational length (~80-90  $\mu\text{g}/\text{kg}/\text{day}$ )
- Decreased litter size; total and live pups (~80-90  $\mu\text{g}/\text{kg}/\text{day}$ )
- Reduced number of litters (~80-90  $\mu\text{g}/\text{kg}/\text{day}$ )
- Increased incidence of undescended testes; developmental delay; (~80-90 and 30  $\mu\text{g}/\text{kg}/\text{day}$ , not statistically significant)
- Accelerated acquisition of puberty – females (~80-90 and 30  $\mu\text{g}/\text{kg}/\text{day}$ )
- Delayed acquisition of puberty – males (~80-90, 30 and 10  $\mu\text{g}/\text{kg}/\text{day}$ )
- Increased female reproductive organ weights and swollen vaginal area (~80-90, 30 and 10  $\mu\text{g}/\text{kg}/\text{day}$ )
- Decreased weight of testes and epididymides in weanlings (~80-90 and 30  $\mu\text{g}/\text{kg}/\text{day}$ )

## 2-Generation Study with BPA in Mice - Strengths

Route of Administration:	Oral dietary (most relevant for human exposure)
Number of Dose Levels:	Six (0.003-600 mg/kg/day)
Number of Animals:	28/sex/dose group/generation
Endpoints Examined:	Multiple
Evaluated for Systemic, Reproductive, and Developmental Toxicity:	Males and females
Positive Control:	17 $\beta$ -estradiol (~0.080-0.090 mg/kg/day)
Number of Negative Control Groups:	Two

# 2-Generation Study with BPA in Mice

This study exceeded the OECD regulatory test guideline No. 416, promulgated in 2001.

The key features of the study were:

- A sensitive strain of mice was exposed to BPA over two parental (F0/F1) and offspring (F1/F2) generations.
- A wide range of doses were examined, from very high to very low, including doses in the “low dose” range. Animals were exposed orally through the diet, which is most relevant for human exposure.
- It was a large-scale study (28 animals/sex/dose level), with in-life lasting over 40 weeks, and appropriate statistical tests and high statistical power to detect effects.
- One additional F1 postwean male/litter was retained to increase the number of F1 offspring (the critical generation) evaluated to adulthood.
- There were nine groups: two concurrent vehicle control groups, one dietary positive control group (0.5 ppm 17 $\beta$ -estradiol) to confirm that the laboratory animals were sensitive to estrogenic effects, and six BPA dose groups, from 0.018-3500 ppm, resulting in intakes of 0.003-600 mg/kg/day BPA.
- Multiple reproductive and developmental endpoints were examined, including a thorough microscopic evaluation of tissues in F1/F2 weanlings and F0/F1 adults.

## 2-Generation Study with BPA in Mice - Results

- Adult systemic toxicity at 50 mg/kg/day (hepatic histopathology) and more pronounced at 600 mg/kg/day (reduced body weights, increased liver and kidney weights, and histopathology).
- Developmental effects at 600 mg/kg/day, including delayed inguinoscrotal testis descent and transient hypoplastic testes in weanling males and slightly delayed acquisition of puberty (PPS) in offspring males, considered not driven by estrogenic activity but likely secondary to systemic toxicity
- No effects on adult reproductive functions (including andrology) or structures (including testes, epididymides, prostate, ovaries, mammary glands, uterus/cervix)
- No low dose BPA effects (0.003 – 5 mg/kg/day)
- No evidence for non-monotonic dose-response curves for any parameters
- Responses to the E2 positive control confirmed the sensitivity of the CD-1 mouse to estrogens and confirmed the findings of the one- and two-generation studies of dietary E2 in mice
- BPA not considered a selective reproductive or developmental toxicant in mice.

# Our Studies Are Robust and Reliable, Because:

- Our multigeneration studies met or exceeded all the requirements of the OECD 416 (and EPA OPPTS 870.3800) reproductive toxicity regulatory testing guidelines, plus enhancements to detect any potential estrogenic effects. The studies were also performed under OECD (and EPA OPPTS) GLP principles and regulations.
- The design, conduct, and reporting of the 2-generation studies with mice were under the guidance and supervision of an international group of expert reproductive and developmental toxicologists from Denmark, Germany, the Netherlands, Sweden, and the United Kingdom, and the European Chemicals Bureau Joint Research Center.
- The protocols, all procedures, data, analyses, and reports from the entire project were subjected to thorough and comprehensive quality assurance auditing, not only by the performing laboratory and the RTI Quality Assurance Unit, but also by an independent, expert third party auditor.
- The two-generation study was reviewed as part of a comprehensive EU risk assessment and described as "the gold-standard, definitive study of the reproductive toxicity of BPA."
- Our BPA work in two species is consistent in the effects we did observe and those effects we did not observe (with comparable systemic, reproductive, and developmental NOAELs in both species).
- Our BPA studies with multiple generations demonstrate reproducibility of our results, and the use of a positive control group confirms the sensitivity of the test species (and strain) to an estrogen.
- FDA recently (April 2009) audited the rat and mouse dietary BPA multigeneration studies and reported no findings for either study.

# Conclusions

- BPA is not a selective developmental or reproductive toxicant in rats or mice
- The reproductive and developmental effects seen at high BPA dietary doses are only observed in the presence of systemic toxicity and are considered secondary to the systemic toxicity observed
- There was no evidence of effects at low BPA doses or non-monotonic dose-responses in any parameter in rats or mice
- The BPA reproductive and developmental effects observed (at high doses) are not consistent with estrogenic activity

# Scientific Evaluation of Bisphenol A (BPA)

F. Jay Murray, Ph.D.

DART Identification Committee Meeting

July 15, 2009

# Introduction and Overview

- Agency Reviews
- Epidemiological Studies
- Animal Studies
- Biological Plausibility
- Species Differences
- Estrogenic Activity

# Agency Reviews of BPA

- NTP Center for the Evaluation of Risks to Human Reproduction (CERHR)
- U.S. Food and Drug Administration
- European Food Safety Authority
- EU Institute for Hlth. & Consumer Protection
- Japanese NIAIST
- Health Canada

# Agency Reviews of BPA

- None reached a conclusion that would support listing BPA
- CERHR: “clear evidence of adverse effects” for “high dose developmental toxicity” in animals
- But, this does not mean that BPA meets the Prop 65 listing standard
  - Effects seen only at high doses associated with systemic toxicity
  - Included studies with both prenatal/postnatal exposure

# Epidemiology

- Studies in humans do not demonstrate that BPA causes developmental or reproductive toxicity
- HID: “[h]uman studies examining the effects of BPA on reproduction are of limited study design and correspondingly limited in their findings.”

# “Conventional” Animal Studies

Well-conducted, conventional studies show:

- BPA is not a selective developmental or reproductive toxicant
- BPA does not cause effects at low doses
- BPA does not produce the reproductive effects observed with  $17\beta$ -estradiol

# “Unconventional, Low-Dose” Animal Studies

- “Inadequate” or “of limited utility” (e.g., small screening studies, parenteral routes, unvalidated methods, insufficient replication, one dose, statistical issues)
- No regulatory agency has relied on a NOAEL from any of these studies
- Do not show BPA is “clearly shown ... to cause”

“... the **failure of BPA to produce reproducible adverse effects via a relevant route of exposure**, coupled with the **lack of robustness** of the many of the low dose studies ... and the **inability to reproduce many of these effects** of (*sic*) any adverse effect strains the credibility of some of these study results. They **need to be replicated** using appropriate routes of exposure, adequate experimental designs and statistical analyses and linked to higher dose adverse effects ...”

-- CERHR

# Biological Plausibility

- Developmental and reproductive effects from BPA are not biologically plausible in humans
  - “First-pass” metabolism following oral exposure to non-estrogenic metabolites
  - Rapid and complete metabolism and elimination prevents ingested BPA from reaching potential target organs

# Species Differences

- Enterohepatic recirculation of BPA is significant in rodents, not humans
- Effects in rodents would over-predict potential responses in humans
- Animal studies using parenteral routes of exposure are not relevant to human hazard identification
- Thus, extrapolation of effects from BPA in rodents to humans is tenuous at best

# Estrogenic Activity

- BPA binds weakly to the estrogen receptor
- No opportunity for estrogenic activity in humans since ingested BPA is converted to inactive metabolites and rapidly excreted
- BPA must compete with estrogenic substances (endogenous and dietary) that have greater affinity for estrogen receptor

# Estrogenic Activity

- Not a basis to list
- Not a reproductive effect *per se*
- But, a potential mechanism that might induce such effects under certain circumstances, which are not present with BPA
- Guidance Criteria do not include it as an endpoint of reproductive toxicity

# Overall Evaluation

- The human data do not support listing.
- The most reliable animal studies show BPA is not a selective developmental or reproductive toxicant
- “Unconventional, low-dose” studies are thought-provoking, but of low utility, conflicting, and insufficient to list BPA (no consistent or compelling evidence)

# Conclusion

- Neither human nor animal studies demonstrate that BPA is “clearly shown to cause” developmental or reproductive toxicity
- Even if the animal studies were sufficient (and they are not), the pharmacokinetic data show that a human hazard is not biologically plausible