TBBPA: Peer Review, NTP 2Year Study

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Albemarle Corporation

- Specialty chemical manufacture
  - Headquarters in Baton Rouge, LA
  - Operations worldwide
- Product line includes flame retardants (FR)
- TBBPA
  - Highest volume brominated FR
  - Use pattern
    - Primary use = epoxy resin circuit boards, covalently bound
    - Secondary use = additive FR in ABS electronic enclosures & starting material for other FRs
    - Historical use = some reports of use in paper or textiles (?)
  - Manufacturing process does not generate methyl bromide

Regulatory History

- US
  - Test rules
    - 1987 Dioxin/Furan Analytical Test Rule
      - None of listed PBDD/PBDFs detected at or above the limit quantitation specified by EPA
      - Rankin et al. 1994. Bull. Soc. Chim Belg. 103(5-6):219-233.
    - 1987 Aquatic and Environmental Fate Test Rule
      - Biodegradation, aquatic toxicology and bioconcentration tests
  - Listed on EPA's Toxic Release Inventory in 2000
  - Included in EPA's HPV Program
    - Data Summary & Test Plan submitted in 2001, updates in 2003 and 2005; Includes unpublished data as of those dates
    - Available to NTP on EPA's website
- EU: 2006 risk assessment, not a human health risk, no concern for carcinogenicity

Comments on NTP's Draft Report

- TBBPA's existing database
- Focus on peer-reviewed literature
  - Unpublished data from robust high quality guideline/GLP-compliant studies on the commercial product
- Differences in kinetics/metabolism between doses, rats and humans, in vivo and in vitro
- Wistar Han rat
- Mortality in mice
- *Tp*53 mutation data
- Uterine sectioning
- Missing data

Unpublished Guideline/GLP Data

- NTP draft report relies on peer-reviewed literature; incomplete
- Significant information relevant to TBBPA's toxicology not included
- Unpublished toxicology data is common for industrial chemicals
  - True for TBBPA
  - Robust, guideline/GLP-compliant; raw data available
  - Re1evant to NTP's 90-d and 2 yr studies
  - On file at EPA: data call-ins, test rules, voluntary submissions; available to NTP

Relevant Unpublished Data

- Physical/Chemical properties (2001, 2002)
- Mammalian & genetox data
  - General lack of toxicity
    - Acute (1970s, 1980s); repeated dose via gavage (2002), diet (1972, 1975, 1986), dermal (1979) or inhalation (1975); prenatal developmental (1975, 1985, 2001); two-generation reproduction w/developmental neurotoxicity (2002), in vitro dermal absorption (2005)
    - High NOELs/NOAELs
  - No effect on growth, development or reproduction in robust repeated dose studies at doses equivalent to NTP
  - Lack of mutagenicity: multiple Ames (1976, 1981, 1986), Chrom Ab (2001)
- Recommend inclusion in NTP's report on TBBPA

TBBPA Kinetics and Metabolism (1)

- Low systemic bioavailability (Brady 1979; Haak et al. 2000; Schauer et al. 2006; Kuester et al. 2007; Knudsen et al. 2013 ABST)
  - <5% at 250 mg/kg (Knudsen et al. 2013 ABST)
- Rapidly absorbed, metabolized and eliminated in feces as parent after deconjugation in gut; low potential for accumulation
  - No accumulation after 10 daily oral doses of 20 mg/kg/d (Kuester et al. 2007)
- Plasma, single oral dose (Schauer et al. 2006)
  - Humans (0.1 mg/kg): Parent n.d., only metabolites
  - Rats (300 mg/kg): Parent minimal, mainly metabolites
  - Reports of detection of parent in general public or environmental biota?
- Major plasma metabolites differ, rats & humans (Schauer et al. 2006)
  - Humans (0.1 mg/kg): glucuronide conjugate (high capacity system)
  - Rats (300 mg/kg): sulfate conjugate (low capacity system)

TBBPA Kinetics and Metabolism (2)

- Strain/gender/dose differences in kinetics
- [Parent] blood, single dose
  - F344 m Cmax ~ 0.4 nmol/ml, 20 mg/kg (Kuester et al. 2007)
  - WH fe Cmax ~ 10 nmol/ml, 250 mg/kg (Knudsen et al. 2013 ABST)
  - SD m Cmax ~100 nmol/ml, 300 mg/kg (Schauer et al. 2006)
- Elimination in bile, single dose
  - Slower at 200 mg/kg than 2 or 20 mg/kg in male F344 (Kuester et al. 2007; funded by NTP prior to initiation of 2 yr)
    - "This most likely represents saturation of metabolic and/or transport processes in the liver."
  - Slower by female WH than male SD or F344 (Knudsen et al. 2013 ABST)
- Identity of bile metabolites differ with time and/or strain

Metabolism and Kinetics (3)

- Identity of bile metabolites after single oral dose
  - 0-24 hr m SD 2 mg/kg: mono/di-glucuronide conjugates, glucuroinde-sulfate conjugate (Haak et al. 2000)
    - Sulfate conjugates predominant in plasma m SD 300 mg/kg (Schauer et al. 2006)
  - 0-2 hr m F344 20 mg/kg: mono & di-glucuronide conjugates (Kuester et al 2007)
- Metabolites in blood at higher or after repeated doses have not been studied in any rat strain; knowledge gap
- In vitro studies of hepatic metabolism (Zalco et al 2006; Chignell et al. 2008)
  - Did not utilize hepatic fraction containing sulfotransferases and/or did not include required sulfate conjugation cofactor (PAPS)
  - Not good models for in vivo metabolism
  - Fig. 1, draft report, in need of revision

Metabolism and Kinetics (4)

- Saturation of hepatic metabolism and/or transport
  - Sulfate conjugate major circulating form in SD rats
    - Low capacity system
  - Evidence for saturation based on elimination
    - Bile: 200 mg/kg in male F344 rats (Kuester et al. 2007)
    - Feces: 1000 mg/kg in female WH rats (Knudesen et al. (2013)
- Suggests maximum dose exceeded at all doses of NTP 2 yr (250, 500, 1000 mg/kg)
  - Prompted Knudsen et al. ABST (2013)?
    - Single oral doses of 25, 250, 1000 mg/kg HW fe
- Potential for saturation and change in metabolism/kinetics at the doses used in the NTP study not discussed in draft report
  - Impact on uterine adenocarcinomas?

Wistar Han Background (1)

- 344/N used by NTP for 30 years
  - Change from F344/N considered in 2005 (King-Herbert and Thayer. 2006)
  - Wistar Han adopted at some point after 2006
- TBBPA 2 year: July ‘07 - July ’09; draft report issued 4 years later
  - Primary effect in uterus
- Feb ‘09 NTP announces Harlan Sprague Dawley in lieu of the Wistar Han due to reproductive capacitiy and size issues (http://ntp.niehs.hih.gov)
  - Decision made while TBBPA 2 yr on-going
  - TBBPA 2 yr study continued

Wistar Han Background (2)

- King-Herbert et al. 2010
  - Wistar Han rat initially chosen as default rat to replace F344
  - Common default considered useful for all tests
    - Default = model initially considered when evaluating a test compound or chemical (pg 181)
  - Wistar Han discontinued as default, but some chronic inhalation studies continued because subchronic studies completed in that rat
    - Opposite choice made for substitution of Wistar Han for F344 in TBBPA 2 year study

Wistar Han Rat (3)

- F344/N used for NTP's 90-d study on TBBPA; Wistar Han rats in 2 year bioassay
- Highly unusual; inconsistent with later NTP policy; insufficient explanation for change in draft report
- Discussion needed in report
  - Dose selection for 2 yr study
  - Selection of gavage as route of administration
  - NTP's decision re F344/N 90D to Wistar Han 2 yr
  - NTP's discontinuance of the Wistar Han
  - NTP historical control data in Wistar Han
  - Possible association of rat strain and the observed uterine adenocarcinomas
    - Reproductive issues led to discontinuance of Wistar Han at NTP
    - Lack of evidence for effect on reproduction/reproductive tract in repeated dose studies w/TBBPA in SD and F344 rats; Neg mutag

Mouse Mortality at High Dose

- TBBPA, 1000 mg/kg/d, survival to 2 yr termination
  - 12 of 50 male mice
  - 4 of 50 female mice
- Discussion needed beyond not using data from high dose
  - Deaths appear associated with gavage based on pathology
    - Impact of 10 ml/kg dosing volume?
  - Pg 69, deaths may be associated with “gastrointestinal toxicity”
    - Evidence for toxicity versus physical from gavage?
    - Factors other than test material (change in personnel?)

Missing Data and Other

- Info on manufacturing process & uses outdated
- Missing data on food consumption
- Numerical values for relative organ weights erroneous for rats and mice at 3 mth and 2 yr
- 3 prenatal developmental (PND) studies
- Experimental design for *Tp*53 mutation data
- Uterine sectioning: transverse & longitudinal
- Speculation on endocrine disruption
  - In vivo data contradicts; evidence not apparent in repeated dose, PND and reproduction studies; in vitro studies of various endpoints do not account for metabolism

Summary

- Updates on manufacture and use pattern, include relevant unpublished toxicology data, revision of kinetics and metabolism needed
- Addition of missing data and information
  - NTP's discontinuance of the Wistar Han
  - Decision to change strains between 90 d and 2 yr
- Further information/discussion
  - Impact of kinetics/metabolism
  - Selection of rat strain, route of administration, doses
  - WH historical control data from NTP studies
  - Possible association of rat strain and the observed uterine adenocarcinomas