DEMONSTRATION OF LARGE MARGIN OF EXPOSURE FOR TBBPA - ASSESSMENT OF TBBPA CANCER AND NON-CANCER EFFECTS AND IMPLICATIONS FOR EXPOSURE

Wikoff D[1*], Thompson C[2], Perry C[1], White M[1], Borghoff S[3], Fitzgerald L[1], Haws LC[1]

ToxStrategies Inc., [1]Austin, TX, USA [2]Houston, TX, USA [3]Cary, NC, USA

Introduction

Available regulatory and health-based assessments quantitatively characterizing TBBPA toxicity and exposure in margin of exposure evaluations rely solely on data for non-cancer endpoints, as there were no data characterizing carcinogenicity at the time that these assessments were conducted. Several of these assessments took similar approaches and reported similar findings; acceptable MOE values were obtained, regardless of exposure scenario and receptor[1,2,3]. A number of relevant toxicity studies have become available since these assessments, most notably, the release of carcinogenicity generated by the National Toxicology Program[4]. As such, the objective of this study was to quantitatively characterize cancer and non-cancer toxicity data for TBBPA. A second objective was to quantitatively characterize potential consumer exposures to TBBPA. These data were then used to conduct a margin of exposure (MOE) assessment for TBBPA. It is anticipated that the exposure estimates, along with the toxicity values described herein, should be informative for risk assessors and regulators interested in characterizing human health hazards associated with TBBPA.

Materials and methods

Quantitative characterization of toxicity:

Toxicity was quantitatively characterized by developing points of departure (POD) for use in MOE. PODs were developed for both cancer and non-cancer endpoints associated with chronic, oral exposure:

- *Selection of toxicity data:* Relevant peer-reviewed and select unpublished *in vivo* studies in mammalian species were reviewed and evaluated for determination of a critical dataset based on Klimisch scores[5] and in particular, considerations for use of multiple dose levels, repeated dosing, and relevant route of exposure. The most robust and sensitive cancer and non-cancer findings from these studies were selected for development of POD values.
- *Dose-response modeling and development of POD values:* The U.S. EPA's Benchmark Dose Software (BMDS) v. 2.4 was used to conduct dose-response modeling on the selected cancer and non-cancer datasets. A benchmark response (BMR) of 10% extra risk was used to obtain benchmark dose (BMD_{10}) values along with the 95% lower confidence limits ($BMDL_{10}$) for dichotomous datasets, whereas the BMR was set to 1 standard deviation in order to obtain (BMD_{1SD}) and ($BMDL_{1SD}$) values for continuous datasets. $BMDL_{10}$ and $BMDL_{1SD}$ were utilized as the POD values.

Selection of media concentration data and estimates of potential exposure:

Exposure to TBBPA was quantitatively characterized by deriving intake estimates associated with oral exposures to food/diet, breast milk consumption, drinking water, and soil/dust ingestion. Two different types of intake estimates were calculated: for evaluation of non-cancer endpoints, an average daily dose (ADD) was generated; for evaluation of cancer endpoints, a lifetime average daily dose (LADD) was generated. Media concentrations were selected from data published in the peer review literature; studies were selected based on study quality, adequate descriptions of methodology, and representativeness of chronic exposure. Central tendency and upper bound exposure scenarios were evaluated for each; these scenarios reflect exposures from the most plausible scenario for the general consumer population (central tendency) and a plausible upper-end for the general consumer population (upper bound). Standard regulatory equations and exposure parameters were utilized to calculate ADD and LADD.

Margin of exposure calculations:

The margin of exposure (MOE) estimates were calculated for cancer and non-cancer endpoints using the following equations:

MOE_{cancer} = POD_{cancer} (mg/kg-day) / LADD (mg/kg-day)
$MOE_{non\text{-}cancer}$ = $POD_{non\text{-}cancer}$ (mg/kg-day) / ADD (mg/kg-day)

Results and discussion

Quantitative characterization of toxicity:

Approximately twenty studies were thoroughly reviewed and considered for use as critical studies in the development of toxicity values for TBBPA. Data from the NTP Toxicological Review on TBBPA[4] were selected for characterization of toxicity as this study was determined to be of highest quality and relevance. In this study, rats and mice of both sexes were exposed via oral gavage to 0, 250, 500, and 1000 mg/kg-day of TBBPA for two years. Endpoints evaluated included body weight, survival, general clinical observations, neoplastic lesions, and non-neoplastic lesions, thus generating a robust dataset based on a chronic duration of exposure (important qualitative for consideration in the quantitative characterization of toxicity in humans). The NTP also conducted a 13-week study that evaluated many of the same endpoints as the 2-year bioassay and also included an evaluation of thyroid hormones as part of a clinical chemistry panel. Endpoints identified in the NTP report with significant, positive/increasing dose-response relationships were carried forward for consideration in the quantitative characterization of cancer and non-cancer point endpoints.

TBBPA was associated with an increased incidence of uterine tumors in Wistar Han rats (classified as clear evidence by NTP) and an increase in the incidence of hepatoblastoma in male B6C3F1/N mice (classified as equivocal evidence by NTP). The combined incidence of uterine tumors (adenomas, adenocarcinomas, and malignant mixed Müllerian tumors) in female rats was modeled using a multistage model to provide the best fit (lowest Akaike information criterion; p-value = 0.75). These efforts yielded a BMD_{10} value of 195.3 mg/kg-day and a $BMDL_{10}$ value of 126.6 mg/kg-day. Further evaluation of hepatoblastoma data revealed lack of a clear dose-response relationship and only marginal significance for hepatoblastomas in male mice, and no treatment related effect was observed for the combined incidence of hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas (neoplasms considered to represent a biological and morphological continuum). Thus, liver tumors were not further considered as a critical endpoint, and the $BMDL_{10}$ value of 126.6 was thus determined to be the POD for cancer-based endpoints.

For non-cancer-based PODs, several non-neoplastic findings were considered: forestomach lesions, uterine hyperplasia, rete ovarian cysts, renal tubule cytoplasmic alterations, hepatic foci, and decreased T4. Each dataset was critically reviewed to determine if the effects were (1) adverse, (2) biologically plausible, and (3) relevant to humans. Because several of the datasets were not determined to meet theses criterion, only uterine hyperplasia, rete ovarian cysts, and forestomach lesions were selected for quantitative characterization of non-cancer toxicity. BMD modeling was for each remaining dataset to obtain BMD_{10} and $BMDL_{10}$ values. These candidate POD values were arrayed and further evaluated. The PODs associated with forestomach lesions were the lowest. This endpoint was associated with questionable relevance to humans given that humans do not have a forestomach. However, both humans and rodents have a glandular stomach, and no TBBPA-induced lesions were reported the glandular stomachs of mice or rats in the 2-yr bioassay. As such, it was determined that the rat uterus was the most sensitive target organ in the bioassay, and the POD of 72.8 based on uterine hyperplasia was selected for non-cancer endpoints.

Selection of media concentration data and estimates of potential exposure:

The large amount of data available characterizing concentrations of TBBPA in food/diet, breast milk, water, and soil/dust were reviewed; studies were selected (Table 1) based on considerations for study quality and relevance, representativeness of chronic consumer exposure, as well as consistency of the data relative to other studies. No preference was given to the country of location where samples were obtained; however, the location and type of samples collected were considered relative to the media type and representativeness of consumer exposure. The use of non-detect data in the analysis and interpretation of such were also carefully considered given that TBBPA was often reported as non-detect.

Table 1. Media concentrations used in the exposure assessment.

Media	Central Tendency	Upper-Bound	Units	Ref
$C_{Milkfat}$	0.0001	0.00128	mg/kg	Shi et al 2013[a,6]
$C_{Soil/Dust}$	0.11	0.46	mg/kg	Harrad et al (2010)[b,7]
C_{DW}	0.00000096	0.000001008	mg/L	Harrad et al 2009[c,8]
Total Dietary Intake	0.000000256	0.00000028	mg/kg-d	Shi et al (2009) [d,9]

[a] Median and 95th percentile, respectively; concentrations are lipid adjusted; % lipid accounted for in the intake calculations.
[b] Median and 95th percentile concentrations, respectively.
[c] Mean and 95th percentile values were derived assuming a normal distribution across the average of the nine lakes and use in the central tendency and upper-bound scenarios, respectively.
[d] Medium bound intake used for central tendency, upper-bound intake used for upper-

Resulting lifetime average daily dose estimates (LADD) are presented in Table 2. Exposure to TBBPA via soil/dust ingestion was the largest contributor, followed by dietary intake (includes both exposure via breast milk and foodstuffs), and to a lesser extent, exposure via drinking water. Average daily dose estimates (ADD), Table 3, varied by scenario and receptor. In infants, the soil/dust pathway was the exposure route that contributed the most to the overall ADD for the Central Tendency scenarios, whereas the percent contribution of exposure via breast milk was significantly greater in the Upper Bound scenario. In young children, older children, and adults, the exposure estimates were driven by dietary intake in the Central Tendency scenario, but by soil/dust exposures in the Upper Bound scenario.

Margin of Exposure

Very large margins of exposure (>7,000,000) were observed for all endpoints, receptors, and scenarios evaluated (Tables 2 and 3). These estimates indicate a low level of health concern.

Mode of Action and Relevance to Humans

It is notable that the critical endpoints selected for the characterization of cancer and non-cancer toxicity were both in the uterus (uterine hyperplasia and uterine tumors). Available data indicate that it is unlikely for TBBPA to act through a genotoxic or mutagenic pathway to elicit the adverse effects observed. Rather, the data suggest that the toxicities observed at high doses may potentially be the result of disruption of endocrine parameters. A number of studies in the literature have reported associations between exposure to TBBPA and endocrine-related effects. While these data may be suggestive of a potential underlying mode of action (MOA) associated with the development uterine lesions, additional research is needed to fully characterize the relevance of such to human exposures. Even the lowest dose used in the NTP study (250 mg/kg-day) is more than five orders of magnitude higher than the highest estimates of exposure generated in this study. Without data characterizing the same endpoints at lower, more environmentally relevant doses, it is often difficult to make such extrapolations; and in particular, it is difficult to differentiate which effects are relevant to human exposure versus which effects may be due to the impact of high doses on physiological function and saturation of protective mechanisms.

Table 2. Intake (LADD) and cancer-based MOE

Route of Exposure	Intake (mg/kg-day)	
	Central Tendency	Upper-Bound
Total Dietary Intake[a]	1.6E-07	2.5E-07
Drinking Water	5.0E-09	1.6E-08
Soil/Dust	5.4E-08	3.7E-07
Total Dose	2.2E-07	6.4E-07
MOE[b]	**5.8E+08**	**2.0E+08**

[a] Includes breast milk and food consumption as appropriate to the receptor.
[b] Calculated using a POD of 126.6 mg/kg-day.

Table 3. Intake (ADD) and non-cancer based MOE

Scenario/Route	Infant (0-12 mo)	Child	Adolescent	Adult
Central Tendency Scenario				
Total Dietary Intake	6.6E-07	9.7E-07	3.6E-07	2.3E-07
Drinking Water	--	1.8E-08	1.0E-08	1.4E-08
Soil/Dust	1.6E-06	2.2E-07	8.1E-08	7.9E-08
Total ADD	2.3E-06	1.2E-06	4.6E-07	3.2E-07
MOE	**3.21E+07**	**6.03E+07**	**1.60E+08**	**2.25E+08**
Upper Bound Scenario				
Total Dietary Intake	6.5E-06	1.1E-06	4.0E-07	2.5E-07
Drinking Water	--	5.6E-08	3.6E-08	4.3E-08
Soil/Dust	3.1E-06	2.6E-06	9.6E-07	3.3E-07
Total ADD	9.6E-06	3.7E-06	1.4E-06	6.2E-07
MOE	**7.61E+06**	**1.98E+07**	**5.23E+07**	**1.17E+08**

Acknowledgements
This work was funded by the North American Flame Retardant Alliance (NAFRA) Panel of the American Chemistry Council (ACC). The funders were given the opportunity to review the draft abstract; the purpose of such review was to allow input on the clarity of the science presented but not in interpretation of the research findings. The researchers' scientific conclusions and professional judgments were not subject to the funders' control; the contents of this abstract reflect solely the view of the authors.

References:
1. Health Canada/ Environment Canada. 2013. Screening Assessment Report: Phenol, 4,4'-(1-methylethylidene) bis[2,6-dibromo- (Chemical Abstracts Service Registry Number 79-94-7), Ethanol, 2,2'-[(1-methylethylidene)bis[(2,6-dibromo-4,1-phenylene)oxy]]bis (Chemical Abstracts Service Registry Number 4162-45-2), Benzene, 1,1'-(1methylethylidene)bis[3,5-dibromo-4-(2- propenyloxy)- (Chemical Abstracts Service Registry Number 25327-89-3).
2. European Union (EU). 2006. Risk Assessment Report: 2,2',6,6'-TETRABROMO-4,4'-ISOPROPYLIDENEDIPHENOL (TETRABROMOBISPHENOL-A or TBBP-A), Part II – Human Health. Volume 63.
3. European Food Safety Administration (EFSA). 2011. *EFSA Journal*. **9**(12): 2477.
4. National Toxicology Program (NTP). 2013. NTP Technical Report on the toxicology studies of tetrabromobisphenol A (CAS no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:Wi(Han)] rats and B6C3F1/N mice. NIH Publication no. 14-5929.
5. Klimisch HJ, Andreae M, Tillmann U. (1997). *Regul Toxicol Pharmacol*. **25**(1): 1-5.
6. Shi Z, Jiao Y, Hu Y, Sun Z, Zhou X, Feng J, Li J, Wu Y. (2013). *Sci Total Environ*. **452-453**: 10-18.
7. Harrad S, Goosey E, Desborough J, Abdallah M, Roosens L, Covaci A. (2010). *Environ Sci Technol*. **44**: 4198-4202.
8. Harrad S, Abdallah MA, Rose NL, Turner SD, Davidson TA. (2009). *Environ Sci Technol*. **43**(24): 9077-83.
9. Shi ZX, Wu YN, Li JG, Zhao YF, Feng JF. 2009. *Environ Sci Technol*. **43**(12): 4314-4319.