Draft Toxicological Review of *t-Butanol*: Mode of Action for Thyroid Follicular Cell Tumors Public Comment: LyondellBasell

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Key Uncertainty Not Considered: Excessively High Top Dose in Mouse Bioassay – Exceeded Limit Dose

- Top doses in NTP (1995) mouse drinking water bioassay:
  - Males: 2070 mg/kg/day; Females: 2110 mg/kg/day
  - Females: only top dose was statistically significant by pair-wise comparison; positive trend test likely dependent on high dose
  - Males: no pair-wise comparison was statistically significant but EPA reported statistical significant trend test.
- EPA guidance on dose selection for animal bioassays - Limit Dose:
  - "The highest dose tested need not exceed 1,000 mg/kg/day." (EPA 870.4300, Combined Chronic Toxicity/Carcinogenicity, 1998)
- Both male and female top dose exceeded Limit Dose by 2-fold, questioning dose-relevance of thyroid tumor findings

Key Uncertainty Not Considered: Excessively High Top Dose in Mouse Bioassay – Exceeded Metabolic Saturation

- TBA is metabolized by CYP450 that is likely subject to saturation in rats and mice, resulting in potential dose-dependent onset of nonlinear toxicokinetics
- Nonlinear toxicokinetics has been demonstrated in mice at dose(s) less than high dose in TBA bioassay (M = 2070; F = 2110 mg/kg/day)

Dose (ip) (mg/kg)	Fold increase (Dose)	TBA AUC (mmol*hr/L)	Fold increase (AUC)
370	1X	28	1X
741	2X	96	3.4X
1482	4X	324	11.6X

Faulkner & Hussain. Res Commun Chem Path Pharmacol 64: 31-39 (1989)

Toxicity Observed Above Metabolic Saturation: Lack of Quantitative Human Relevance

- Changes in toxicokinetics with increasing dose may result "…in important differences between high and low dose levels in disposition of the agent or generation of its active forms. These studies play an important role in providing a rationale for dose selection in carcinogenicity studies." (EPA Cancer RA Guidelines, 2005)
- "Available toxicokinetic data (ADME) should *always* [emphasis added] be taken into account when selecting dose levels for a chronic toxicity or carcinogenicity study… Many toxicokinetic processes influencing absorption, distribution, elimination and metabolic activation or detoxication may become saturated at higher doses, resulting in systemic exposures to parent compound or metabolites that would not be expected in the real life human exposures for which risk assessments are needed." (OECD Guidance Document 116 on the conduct of chronic toxicity and carcinogenicity studies, 2012)

Toxicity Observed Above Metabolic Saturation: Lack of Quantitative Human Relevance

- "Although top dose selection based on identification of inflection points in toxicokinetic nonlinearity may result in study designs that fail to identify traditional target organ or body weight effects, it must be appreciated that metabolic saturation in fact represents an equivalent indicator of biological stress. In this case, the stress is evidenced by appearance of non-linear toxicokinetics rather than appearance of histological damage, adverse changes in clinical chemistry, haematology parameters or decrease in body weight gain." (OECD Guidance Document 116)

Impact of High Dose & Toxicokinetic Considerations on Uncertainty of Mouse Thyroid Tumors

- If 1995 TBA bioassay was designed according to current dose selection guidance of EPA and OECD, mouse thyroid tumors likely would not have emerged as a significant cancer concern.