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Preliminary Materials
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Preliminary Materials for the Integrated Risk Information System (IRIS) Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)

[CASRN 75-65-0]

July 2013

NOTICE

This document is comprised of preliminary materials, consisting of a literature search strategy, evidence tables, and exposure-response arrays. This information is distributed solely for the purpose of pre-dissemination review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

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PREFACE

This document presents the draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays for *tert*-butyl alcohol (henceforth referred to as *tert*-butanol) prepared under the auspices of EPA's Integrated Risk Information System (IRIS) Program. This material is being released for public viewing and comment prior to a public meeting, providing an opportunity for the IRIS Program to engage in early discussions with stakeholders and the public on data that may be used to identify adverse health effects and characterize exposure-response relationships.

The draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays are responsive to the National Research Council (NRC) 2011 report *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde*. The literature search strategy, which describes the processes for identifying scientific literature, screening studies for consideration, and selecting studies for inclusion in evidence tables, is responsive to NRC recommendations regarding systematic review of the scientific literature. In addition, NRC recommendations for standardized presentation of key study data are addressed in the preliminary evidence tables and preliminary exposure-response arrays.

EPA welcomes all comments on the draft literature search strategy, preliminary evidence tables, and preliminary exposure-response arrays, such as remarks on the following:

- the clarity and transparency of the materials;
- the approach for identifying pertinent studies;
- the selection of studies for data extraction to preliminary evidence tables and exposure-response arrays;
- any methodological considerations that could affect the interpretation of or confidence in study results; and
- any additional studies published or nearing publication that may provide data for the evaluation of human health hazard or exposure-response relationships.

The preliminary evidence tables and exposure-response arrays should be regarded solely as representing the data on each endpoint that have been identified as a result of the draft literature search strategy. They do not reflect any conclusions as to hazard identification or dose-response assessment. After obtaining public input and conducting additional study evaluation and data integration, EPA will revise these materials to support the hazard identification and dose-response assessment in a draft Toxicological Review.

1. DRAFT LITERATURE SEARCH STRATEGY

1.1. Literature Search and Screening Strategy for *tert*-Butanol

The overall literature search approach is shown graphically in Figure 1-1. The initial chemical-specific search was conducted in four online scientific databases in December, 2012, and January, 2013, using the keywords and limits described in Table 1-1. After electronically eliminating duplicates from the citations retrieved through these databases, 2,907 unique citations were identified. An additional seven citations were obtained using additional search strategies described in Table 1-2.

The resulting 2,410 citations were screened using the title, abstract, and/or full text for pertinence to examining the health effects of *tert*-butanol exposure. A total of 2,226 references were identified as not being pertinent and were excluded from further consideration (see Figure 1-1 for the exclusion categories). A total of 106 references were identified as primary sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays (see Section 1.2.1). A total of 60 references were considered pertinent, but not as primary sources of health effects data (e.g., ADME studies), and kept as additional resources for development of the Toxicological Review (see Section 1.2.2). A total of 18 references did not provide enough material to evaluate pertinence (e.g., foreign language), and were reserved for further possible review (see Section 1.2.3).

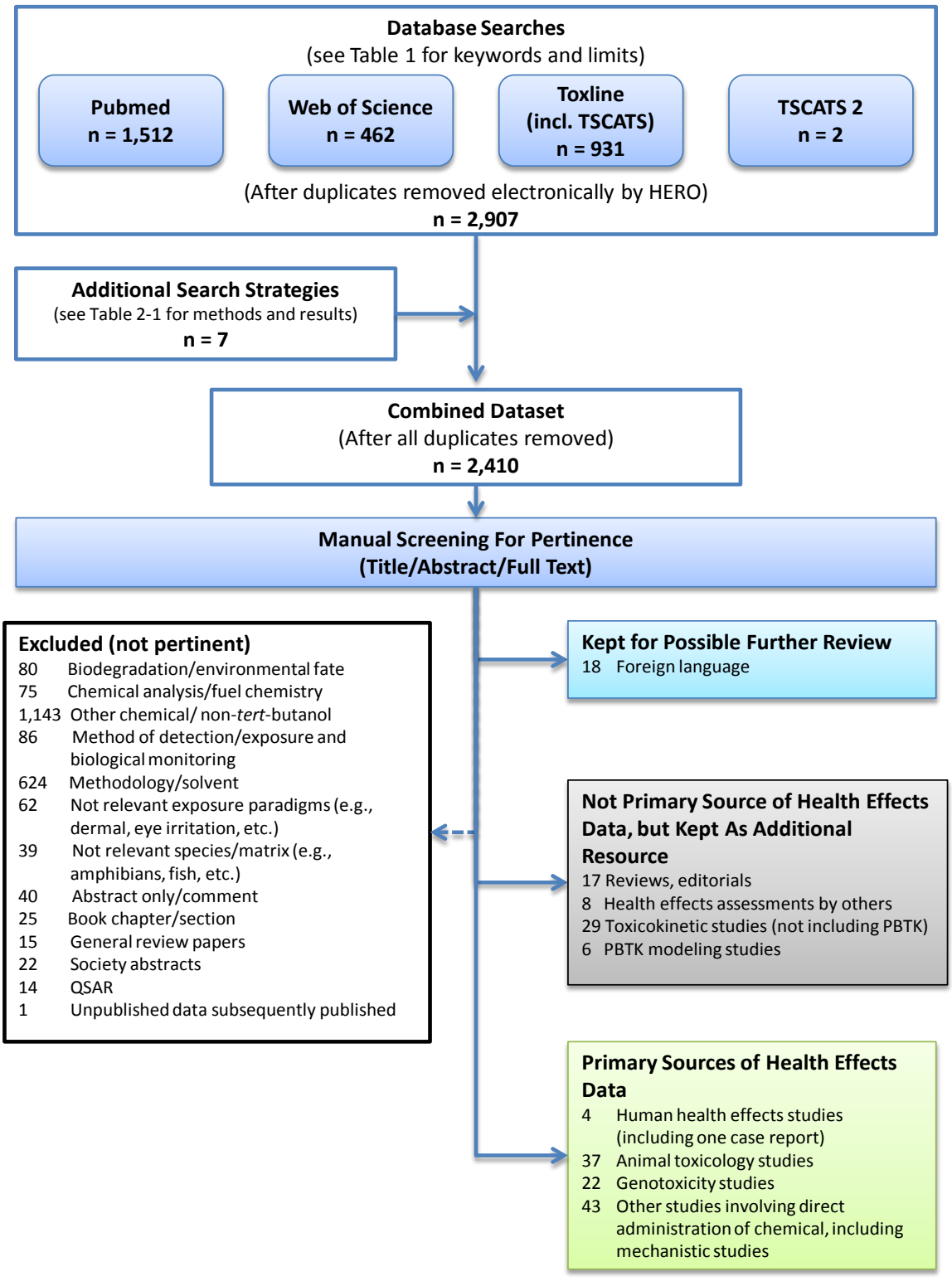

Figure 1-1. Literature search approach for *tert*-butanol.

Table 1-1. Database search strategy for *tert*-butanol

Database (Search Date)	Keywords	Limits
PubMed (12/20/2012)	*t-butanol OR 75-65-0[rn] OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR tert-butanol OR "tert-butyl alcohol" OR tert-butyl alcohol[mesh]*	None
Web of Science (12/20/2012)	Topic = *(t-butanol OR 75-65-0 OR "t-butyl hydroxide" OR "2-methyl-2-propanol" OR "trimethyl carbinol" OR "t-butyl alcohol" OR "tert-butanol" OR "tert-butyl alcohol")*	Refined by: Research Areas = (cell biology OR respiratory system OR microscopy OR biochemistry molecular biology OR gastroenterology hepatology OR public environmental occupational health OR oncology OR physiology OR cardiovascular system cardiology or toxicology OR life sciences biomedicine other topics OR hematology OR pathology OR neurosciences neurology OR developmental biology)
Toxline (includes TSCATS) (1/11/2013)	*t-butanol OR 75-65-0 [rn] OR t-butyl hydroxide OR 2-methyl-2-propanol OR trimethyl carbinol OR t-butyl alcohol OR tert-butanol OR tert-butyl alcohol OR tert-butyl alcohol*	Not PubMed
TSCATS2 (1/4/2013)	75-65-0	None

Table 1-2. Summary of additional search strategies for *tert*-butanol

Approach used	Source(s)	Date performed	Number of additional citations identified
Manual search of citations from reviews	Review article: McGregor, D. (2010). Tertiary-butanol: A toxicological review. Crit Rev Toxicol 40(8): 697-727.	1/2013	5 citations
	Review article: Chen, M. (2005). Amended final report of the safety assessment of t-butyl alcohol as used in cosmetics." Int J Toxicol 24(2): 1-20.	1/2013	2 citations
Manual search of citations from reviews conducted by other international and federal agencies	IPCS (International Programme on Chemical Safety) (1987a). Butanols: Four isomers: 1-butanol, 2-butanol, tert-butanol, isobutanol [WHO EHC]. Geneva, Switzerland: World Health Organization.	1/2013	None
	OSHA (Occupational Safety & Health Administration). (1992). Occupational safety and health guideline for tert-butyl alcohol. Cincinnati, OH: National Institute for Occupational Safety and Health.	1/2013	None

1.2. List of References Based on Search Strategy for *tert*-Butanol

Citations for excluded references are not listed here, but can be found on the Health and Environmental Research Online (HERO) Web site (http://hero.epa.gov/*tert*-Butanol).

1.2.1. Primary Sources of Health Effects Data

Data from citations in **bold** are displayed in Section 2. See Section 2.1 for a description of the process of selecting these studies for evidence tables and exposure-response arrays.

Human health effects studies

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Animal toxicology studies

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3) ARCO (ARCO Chemical Company). (1992). Initial submission: letter submitting preliminary results from subchronic toxicity studies of tertiary butyl alcohol in rats and mice dated 10/14/92 and attachments. (88930000018). Newton Square, PA: Arco Chemical Company.

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13) Faulkner, TP; Wiechart, JD; Hartman, DM; Hussain, AS. (1989). The effects of prenatal tertiary butanol administration in CBA/J and C57BL/6J mice. Life Sci 45: 1989-1995.

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17) GSRI (Gulf South Research Institute). (1979a). Repeated dose test of t-butanol (c55367) in B6C3F1 mice and Fischer 344 rats with cover letter dated 031594. (86940000173). Orleans Parrish, LA.

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30) NTP (National Toxicology Program). (1997). NTP technical report on toxicity studies of t-butyl alcohol (CAS no 75-65-0) administered by inhalation to F344/N rats and B6C3F1 mice. Research Triangle Park, NC.

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16) Sgambato, A; Iavicoli, I; De Paola, B; Bianchino, G; Boninsegna, A; Bergamaschi, A; Pietroiusti, A; Cittadini, A. (2009). Differential toxic effects of methyl tertiary butyl ether and tert-butanol on rat fibroblasts in vitro. Toxicol Ind Health 25: 141-151.

17) Tang, G; Wang, J; Zhuang, Z. (1997). [Cytotoxicity and genotoxicity of methyl tert-butyl ether and its metabolite to human leukemia cells]. Zhonghua Yufang Yixue Zazhi 31: 334-337.

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2. PRELIMINARY EVIDENCE TABLES AND EXPOSURE-RESPONSE ARRAYS

2.1. Data Extraction: Preparation of Preliminary Evidence Tables and Exposure-Response Arrays

The 106 references identified as primary sources of health effects data were considered for data extraction to evidence tables and exposure-response arrays. References were first collated with respect to exposure route, exposure duration, and type of endpoint, to identify those most pertinent for evaluating the human health effects from chronic oral or inhalation exposure to *tert*-butanol. As a result, data from 73 studies with one or more of the following characteristics were not extracted into evidence tables or exposure-response arrays:

- The study involved dermal exposure;
- The study only involved acute or short-term exposures (less than 90 days/13 weeks), and it was not conducted in the context of immune, neurological, developmental, or reproductive toxicity;
- The data in the study only included endpoints related to possible mechanisms of toxicity;
- The study's endpoints did not exhibit responses in any of the 106 available references.

Data from the 33 remaining references were prepared in preliminary evidence tables. No studies were excluded based on study quality considerations, so as to allow for public input on methodological considerations that could affect the interpretation of or confidence in each study's results. With regard to noncancer effects, health effect endpoints that were consistently affected in chronic or subchronic studies were included in the evidence tables. All data demonstrating carcinogenic effects were included. Supporting data that provide mechanistic information for each selected endpoint were also included. For each included endpoint, all studies reporting data on that endpoint are included regardless of the reported level or statistical significance of the response. The tables are arranged in the order from the health effect with the most data to health effect with the least data, with carcinogenic effects grouped with non-carcinogenic effects in the same tissue or system. For each endpoint, the studies are presented beginning with subchronic studies followed by chronic exposures. The evidence table for all reported genotoxicity endpoints follows. The information in the preliminary evidence tables is displayed graphically in preliminary exposure response arrays. In these preliminary arrays, the doses are labeled based only on statistical significance as determined by the study's authors, without consideration of biological significance.

As a consequence, endpoints for which the study's authors did not report results of statistical significance tests were not included in these preliminary arrays.

2.2. Kidney Effects

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol

Reference and study design	Results
Kidney weight (percent change as compared to control)	
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	**Males** Dose (mg/kg-d) / Left absolute Weight / Left relative Weight / Right absolute Weight / Right relative Weight 0 / 0 / 0 / 0 / 0 64 / +6 / +8 / +6 / +8 160 / +9 / +14* / +6 / +11* 400 / +12* / +14* / +14* / +17* 1,000 / +18* / +28* / +20* / +31* **Females** Dose (mg/kg-d) / Left absolute Weight / Left relative Weight / Right absolute Weight / Right relative Weight 0 / 0 / 0 / 0 / 0 64 / −1 / −2 / +2 / 0 160 / 0 / 0 / +1 / 0 400 / +3 / +2 / +4 / +2 1,000 / +4 / 0 / +7 / +2
NTP (1995) F344/N rat; 10/sex/treatment Drinking water 0, 2.5, 5, 10, 20, 40 mg/mL M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	**Males** / **Females** Dose (mg/kg-d) / Absolute Weight / Relative Weight / Dose (mg/kg-d) / Absolute Weight / Relative Weight 0 / 0 / 0 / 0 / 0 / 0 230 / +12* / +19* / 290 / +19* / +17* 490 / +17* / +26* / 590 / +16* / +15* 840 / +16* / +32* / 850 / +29* / +28* 1,520 / +26* / +54* / 1,560 / +39* / +40* 3,610 / All dead / All dead / 3,620 / +36* / +81*

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	**Males** / **Females** Dose (mg/kg-d) / Absolute weight / Relative weight / Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 / 0 350 / +1 / +1 / 500 / 0 / −3 640 / +3 / +2 / 820 / −3 / −1 1,590 / +2 / +8 / 1,660 / +1 / 0 3,940 / +6 / +22* / 6,430 / +6 / +15* 8,210 / 0 / +48* / 11,620 / +12* / +35*
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	**Males** / **Females** Dose (mg/kg-d) / Absolute weight / Relative weight / Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 / 0 90 / +4 / +8 / 180 / +8* / +14* 200 / +11 / +15* / 330 / +18* / +21* 420 / +7 / +20* / 650 / +22* / +42* Only animals sacrificed at 15 months were evaluated for organ weights.
Histopathology	
Acharya et al. (1997; 1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	↑ tubular degeneration, degeneration of the basement membrane of the Bowman's capsule, diffused glomeruli, and glomerular vacuolation (no incidences reported) ↓ kidney glutathione (~40%)*
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	There were no changes in kidney histopathology observed.

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results					
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Incidence (severity):					
	Males			**Females**		
	Dose (mg/kg-d)	Mineralization	Nephropathy	Dose (mg/kg-d)	Mineralization	Nephropathy
	0	0/10	7/10 (1.0)	0	10/10 (1.7)	2/10 (1.0)
	230	0/10	10/10 (1.6*)	290	10/10 (2.0)	3/10 (1.0)
	490	2/10 (1.5)	10/10 (2.6*)	590	10/10 (2.0)	5/10 (1.0)
	840	8/10*(1.4)	10/10 (2.7*)	850	10/10 (2.0)	7/10* (1.0)
	1,520	4/10*(1.0)	10/10 (2.6*)	1,560	10/10 (2.0)	8/10* (1.0)
	3,610	4/10*(1.0)	7/10 (1.1)	3,620	6/10 (1.2)	7/10* (1.0)
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	There were no changes in kidney histopathology observed (histopathology data for the 13-week study were not provided)					

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, 10 mg/mL) M: 0, 90, 200, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	Incidence (severity): **Males** Dose (mg/kg-d) / Mineralization (interim) / Mineralization (terminal) / Linear mineralization (terminal) 0 / 1/10 (1.0) / 26/50 (1.0) / 0/50 90 / 2/10 (1.0) / 28/50 (1.1) / 5/50* (1.0) 200 / 5/10 (1.8) / 35/50 (1.3) / 24/50* (1.2) 420 / 9/10* (2.3) / 48/50* (2.2) / 46/50* (1.7) Dose (mg/kg-d) / Renal tubule hyperplasia (extended evaluation) / Transitional epithelium hyperplasia / Nephropathy severity 0 / 12/50 (2.3) / 25/50 (1.7) / 3.0 90 / 16/50 (2.3) / 32/50 (1.7) / 3.1 200 / 14/50 (2.2) / 36/50* (2.0) / 3.1 420 / 23/50* (2.8) / 40/50* (2.1) / 3.3* **Females** Dose (mg/kg-d) / Mineralization[b] Interim / Mineralization[b] Terminal / Inflammation (suppurative) incidence 0 / 10/10 (2.8) / 49/50 (2.6) / 2/50 180 / 10/10 (2.9) / 50/50 (2.6) / 3/50 330 / 10/10 (2.9) / 50/50 (2.7) / 13/50* 650 / 10/10 (2.8) / 50/50 (2.9) / 17/50* Dose (mg/kg-d) / Renal tubule hyperplasia / Transitional epithelium hyperplasia / Nephropathy severity 0 / 0/50 / 0/50 / 1.6 180 / 0/50 / 0/50 / 1.9* 330 / 0/50 / 3/50 (1.0) / 2.3* 650 / 1/50 (1.0) / 17/50*(1.4) / 2.9*

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	No changes in kidney related histopathology observed.[c]
Tumors	
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	**Male** Dose (mg/kg-d) / Renal tubule adenoma[d] / Renal tubule adenoma (multiple)[d] / Renal tubule adenoma or carcinoma[d] 0 / 7/50 / 1/50 / 8/50 90 / 7/50 / 4/50 / 13/50 200 / 10/50 / 9/50* / 19/50* 420 / 10/50 / 3/50 / 13/50 Results do not include the animals sacrificed at 15 months. Females did not develop kidney tumors.
Hard et al. (2011); reanalysis of the slides in the NTP (1995) study (see above)	**Male** Dose (mg/kg-d) / Renal tubule adenoma / Renal tubular adenoma (multiple) / Renal tubule adenoma or carcinoma 0 / 3/50 / 1/50 / 4/50 90 / 9/50 / 3/50 / 13/50* 200 / 9/50 / 9/50 / 18/50* 420 / 9/50 / 3/50 / 12/50*
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	No changes in kidney-related tumors

Table 2-1. Evidence pertaining to kidney effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
Additional studies potentially relevant for informing the mode of action	
Williams and Borghoff (2001) F344 rats; 4/sex Single gavage dose: 500 mg/kg	Males: ↑ binding of *tert*-butanol to α_{2u}-globulin compared to females* Females: no change in binding observed
Takahashi et al. (1993) F344 rats (M); 10/treatment Drinking water (0, 0.25, 0.5, 1, 2 ,4% w/v) 0,230,490, 840, 1,520 or 3,610[a] mg/kg-d 13 weeks	↑ in hyaline droplet accumulation (number and size) with ↑ severity for nephropathy (no incidence data) **Male** Dose (mg/kg-d) / Hyaline droplet severity score / Hyaline crystals severity score / Nephropathy severity score 0 / 1 / 0.5 / 1 230 / 1.5 / 2* / 2* 490 / 2* / 3* / 3* 840 / 2* / 3* / 3* 1,520 / 2* / 3* / 3* 3,610 / >0.25* / >0.25* / 1
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Accumulation of hyaline droplets: **Male** Dose (mg/kg-d) / Hyaline droplet accumulation 0 / 0/10 230 / +[e] 490 / ++ 840 / ++ 1,520 / ++ 3,610 / 0/10 No information provided on females. No results from statistical tests reported.
Hard et al. (2011) Reanalysis of the slides in the NTP (1995) study (see above)	Males: Confirmed accumulation of hyaline droplets increased with increasing dose-levels in 13 week study above. No incidence data available. Females: not evaluated

[a] The high-dose group had an increase in mortality.
[b] Linear mineralization not observed in female rats.
[c] Organs were not weighed in mice during the 2-year study.
[d] Standard & extended evaluation combined.
[e] + or ++ indicated an increased accumulation relative to controls, as reported by the authors; no additional incidence data and no results from statistical tests available.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.

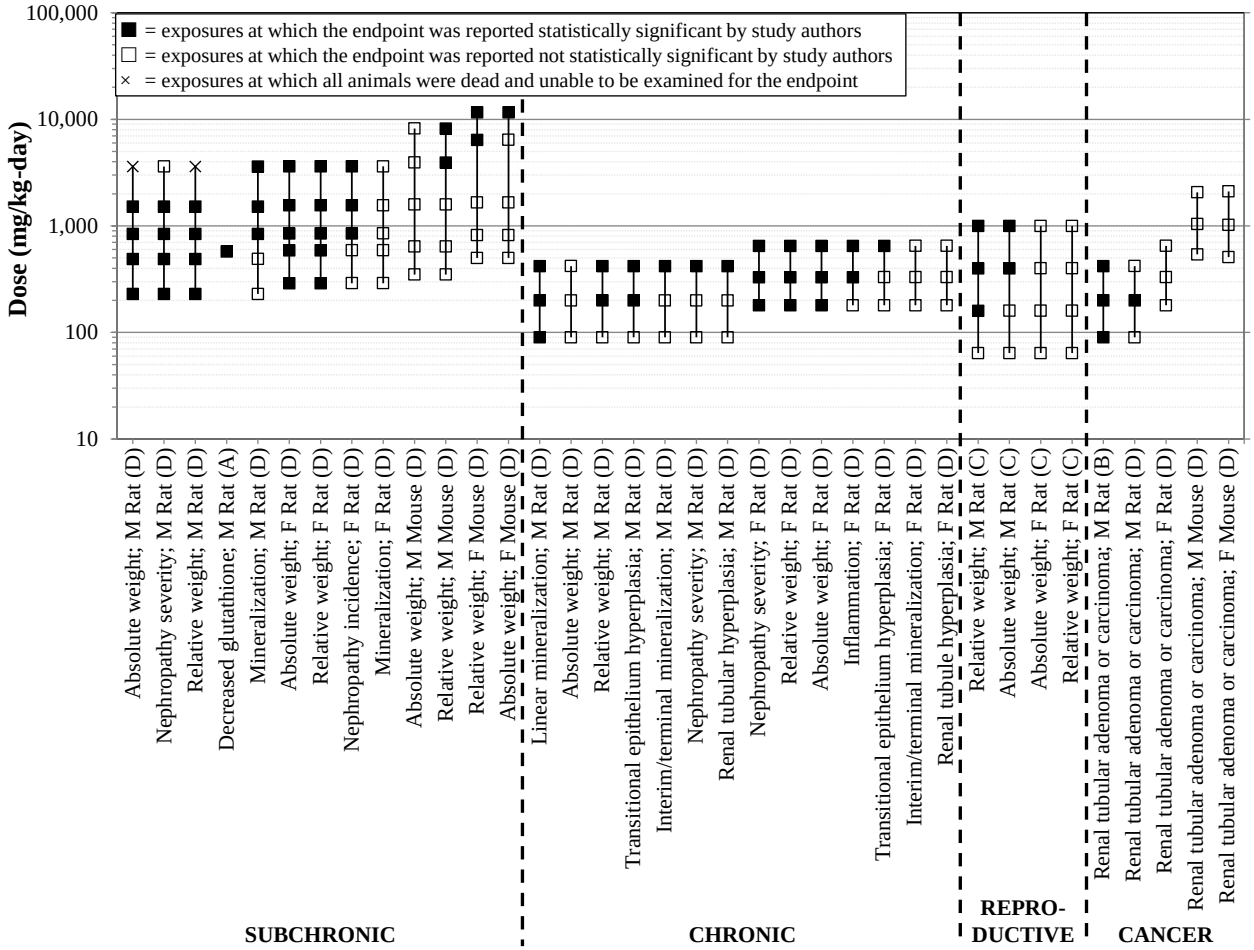
Table 2-2. Evidence pertaining to kidney effects in animals following inhalation exposure to *tert*-butanol

Reference and study design	Results				
Kidney weight (percent change compared to control)					
NTP (1997) F344/N rat; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks		**Males**		**Females**	
	Dose (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	0	0	0	0
	406	+1	+1	−4	−1
	824	−2	−1	0	+1
	1,643	+3	+2	+4	+4
	3,273	+11*	+8*	+2	+2
	6,368	+9.8*	+9*	+4	+9*
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks		**Males**		**Females**	
	Dose (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	0	0	0	0
	406	−6	−4	+1	−3
	824	−1	+3	+5	+9
	1,643	+4	+3	+1	−2
	3,273	−10	−3	0	+7
	6,368	+3	+6	+3	+15*
Histopathology					
NTP (1997) F344/N rat; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks	**Male**				
	Dose (mg/m^3)	Average severity of chronic nephropathy			
	0	1.0			
	406	1.4			
	824	1.4			
	1,643	1.6			
	3,273	1.9			
	6,368	2.0			
	Severity categories: 1= minimal, 2= mild. No results from statistical tests reported				

Table 2-2. Evidence pertaining to kidney effects in animals following inhalation exposure to *tert*-butanol (continued)

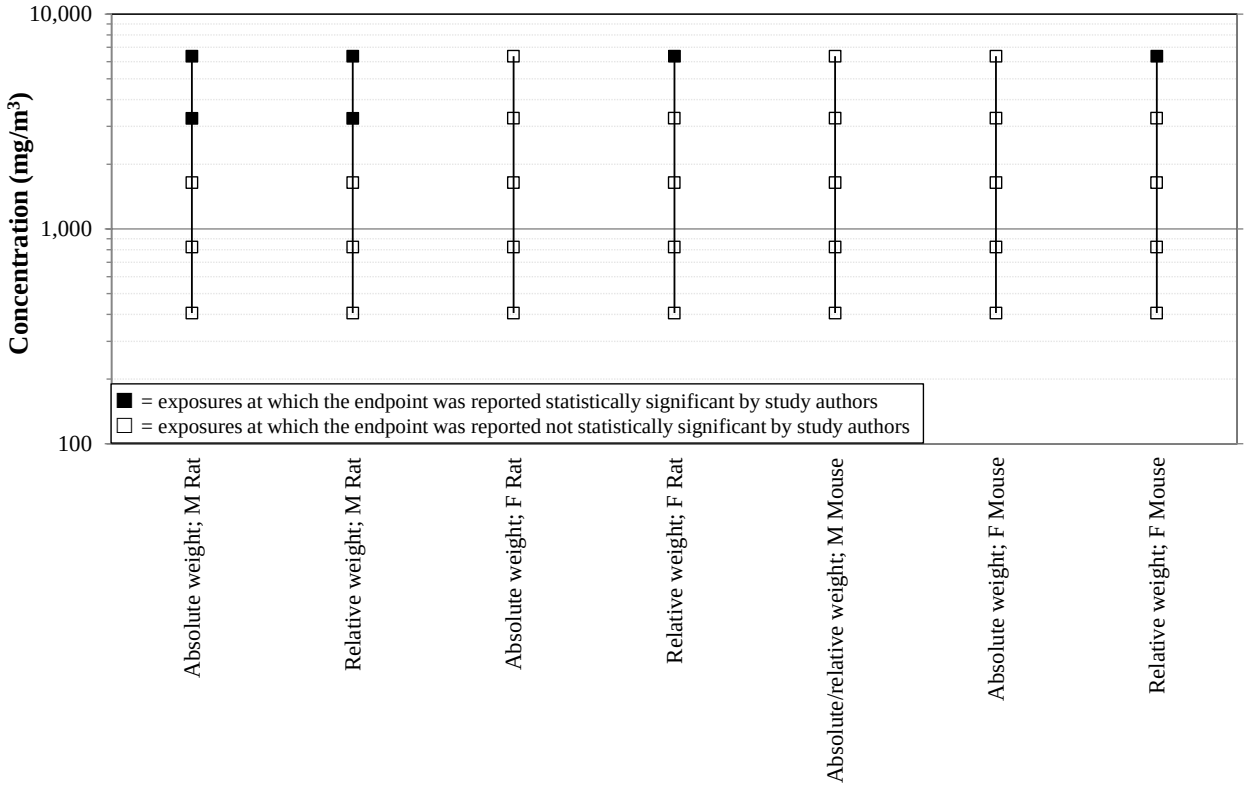
Reference and study design	Results
NTP (1997) $B6C3F_1$ mouse; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks	There were no kidney effects observed.
Additional studies potentially relevant for informing the mode of action	
Borghoff et al. (2001) F344 rat; 5/sex/treatment Analytical concentration:0, 250, 450, 1,750 ppm (0,771, 1,387 or 5,395mg/m^3) 6hr/d 10 days	Males: positive trend for accumulation of protein droplets ($p < 0.05$), significant increase in accumulation of α_{2u}-globulin at 5,394 mg/m^3 as compared to controls (no incidence data provided) Females: No positive staining for α_{2u}-globulin was observed in exposed female rats.

* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.



Sources: (A) Acharya et al. (1997; 1995); (B) Hard et al. (2011)*; (C) Lyondell (2004); (D) NTP (1995)
*: reanalysis of NTP (1995)

Figure 2-1. Exposure response array of kidney effects in rats and mice following oral exposure to *tert*-butanol.



Source: NTP (1997)

Figure 2-2. Exposure-response array of kidney effects in rats and mice following subchronic inhalation exposure to *tert*-butanol.

2.3. Thyroid Effects

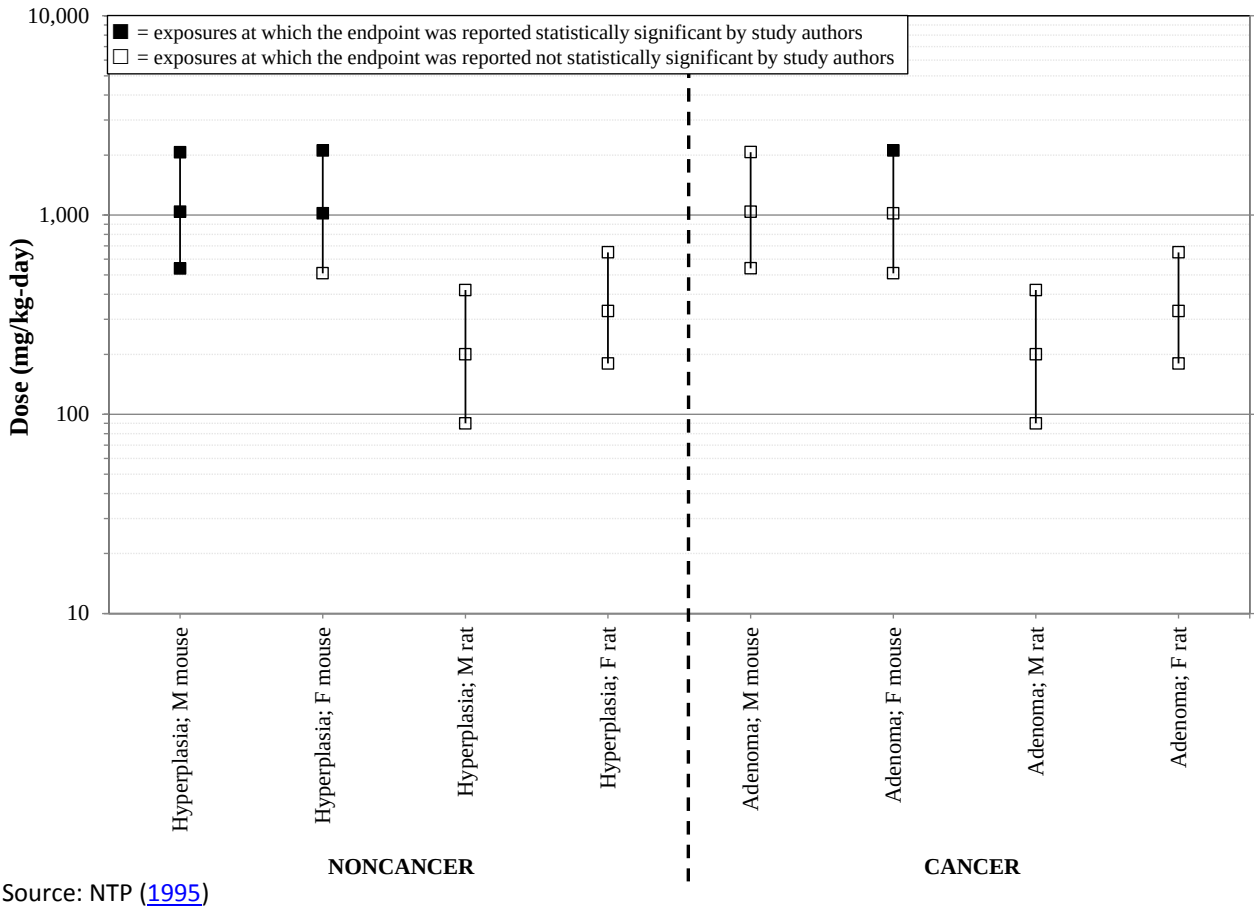
Table 2-3. Evidence pertaining to thyroid effects in animals following oral exposure to *tert*-butanol

Reference and study design	Results			
Follicular cell hyperplasia				
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	Incidence[b]			
	Males		**Females**	
	Dose (mg/kg-d)	Follicular cell hyperplasia	Dose (mg/kg-d)	Follicular cell hyperplasia
	0	3/50	0	0/50
	90	0/49	180	0/50
	200	0/50	330	0/50
	420	0/50	650	0/50
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	Incidence (severity)			
	Males		**Females**	
	Dose (mg/kg-d)	Follicular cell hyperplasia	Dose (mg/kg-d)	Follicular cell hyperplasia
	0	5/60 (1.2)	0	19/58 (1.8)
	540	18/59* (1.6)	510	28/60 (1.9)
	1,040	15/59* (1.4)	1,020	33/59* (1.7)
	2,070	18/57* (2.1)	2,110	47/59* (2.2)
Tumors				
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	Incidence[b]			
	Dose (mg/kg-d)	Follicular cell adenoma	Follicular cell carcinoma	
	Male			
	0	2/50	2/50	
	90	0/49	0/49	
	200	0/50	0/50	
	420	0/50	0/50	
	Female			
	0	1/50	1/50	
	180	0/50	0/50	
	330	1/50	1/50	
	6,500	0/50	0/50	

Table 2-3. Evidence pertaining to thyroid effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results				
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	Incidence				
	Dose (mg/kg-d)	Follicular cell adenoma	Mortality-adjusted rate (%)	Follicular cell carcinoma or adenoma	Mortality-adjusted rate (%)
	Male				
	0	1/60	3.6	1/60	3.6
	540	0/59	0.0	0/59	0.0
	1,040	4/59	10.1	4/59	10.1
	2,070	1/57	5.9	2/57	8.7
	Female				
	0	2/58	5.6	2/58	5.6
	510	3/60	8.6	3/60	8.6
	1,020	2/59	4.9	2/59	4.9
	2,110	9/59*	19.6	9/59*	19.6

[a]There was a significant decrease in survival in the high-dose group.
[b]Results do not include the animals sacrificed at 15 months.
* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.



Source: NTP (1995)

Figure 2-3. Exposure-response array of thyroid follicular cell effects in rats and mice following chronic oral exposure to *tert*-butanol.

2.4. Reproductive, Developmental, and Neurodevelopmental Effects

Table 2-4. Evidence pertaining to reproductive effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Oral	
Billitti et al. (2005) CD-1 male mice; number unclear Gavage, 0, 400, 1,000, 2,000 mg/kg Single dose	no effect on testosterone levels in feces or serum (data not provided) no effect on body weight (statistical tests not reported) ↑ testes weights (averaged weights in the 1,000 and 2,000 mg/kg groups +14% higher relative to controls; $p \leq 0.05$) higher percentage (+7%; $p \leq 0.05$) of sloughing in the seminiferous epithelium in the control animals
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d P0 Males and Females: At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	P0 reproductive effects M: No effects weights of male reproductive organs or sperm observed F: Dose (mg/kg-d): 0 / 64 / 160 / 400 / 1000 Pregnancy index 91.7% / 91.7% / 100% / 100% / 91.7% Live pups/litter *response relative to control* 0 / −9 / −11 / −7 / −33*
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed No significant effect on female estrous cycle (0, −2, −4, 0, +8 % change relative to control)
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	No significant effect on weights of male reproductive organs or sperm observed ↑ length of estrous cycle *Response relative to control*: 0, +5, +5, +5, +6, +28*%

Table 2-4. Evidence pertaining to reproductive effects in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results
Inhalation	
NTP (1997) F344/N rat; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks	No significant effect on weights of male reproductive organs or sperm observed No significant effect on female estrous cycle (0, −4, +2, +4 % change relative to control) Evaluations were only performed for concentrations ≥542 ppm
NTP (1997) B6C3F$_1$ mouse; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks	No significant effect on weights of male reproductive organs or sperm observed No significant effect on female estrous cycle (0, −3, −9, −5 % change relative to control) Evaluations were only performed for concentrations ≥542 ppm

* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Table 2-5. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Oral	
Daniel and Evans (1982) Swiss Webster (Cox) mouse; 15 pregnant dams/treatment Liquid diet (0, 0.5, 0.75, 1.0%, w/v) 0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, 6,677 mg/kg-d GD 6–20	No statistical analysis was conducted on any of these data **Maternal** Percent change compared to control: Dose (mg/kg-d) / Body weight on GD 20 / Body weight gain / Number of litters (% pregnant dams) 0 / 0 / 0 / 11 (77%) 3,324 / +2 / −3 / 12 (80%) 4,879 / −5 / −19 / 8 (53%) 6,677 / −10 / −20 / 7 (47%) **Fetal** Percent change compared to control: Dose (mg/kg-d) / Number of neonates/litter / Fetal body weight on PND 2 0 / 0 / 0 3,324 / −1 / −7 4,879 / −29 / −19 6,677 / −49 / −38 Number of stillborn also increased with dose (3, 6, 14, and 20, respectively), but appropriate denominators not reported so statistical significance of this change could not be calculated. The high dose also caused a delay in eye opening and a lag in weight gain during PND 2–10 (information was only provided in text or figures)
Faulkner et al. (1989) CBA/J mouse; 7 pregnant females in control, 12 pregnant females in treated Gavage (10.5 mmoles/kg twice a day); 0 (tap water), 1,556 mg/kg-d GD 6–18	Maternal results not reported. **Fetal** Percent change compared to control: / Incidence: Dose (mg/kg-d) / Live fetuses/litter / Fetal weight / Sternal variations / Skull variations 0 / 0 / 0 / 4/28 / 1/28 1,556 / −41* / −4 / 7/30 / 3/30 Sternal variations: misaligned or unossified sternebrae Skull variations: moderate reduction in ossification of supraoccipital bone Number of total resorptions (10 resorptions/66 implants in controls, 37/94 implants in treated) and resorptions per litter resorptions per litter (+118%) increased ($p < 0.05$)

Table 2-5. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results
Faulkner et al. (1989) C57BL/6J mouse; 5 pregnant females in controls, 9 pregnant females treated Gavage (10.5 mmoles/kg twice a day) 0 (tap water), 1,556 mg/kg-d GD 6–18	Maternal results not reported. **Fetal** Percent change compared to control: / Incidence: Dose (mg/kg-d) / Live fetuses/litter / Fetal weight / Sternal variations / Skull variations 0 / 0 / 0 / 5/21 / 1/21 1,556 / −58%* / −4 / 9/16 / 7/16 Sternal variations: misaligned or unossified sternebrae Skull variations: moderate reduction in ossification of supraoccipital bone Number of total resorptions (4 resorptions/44 implants in controls, 38/68 implants in treated) and resorptions per litter resorptions per litter (+428%) increased ($p < 0.05$)
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d P0 Males and Females: At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation. F1 Males and Females: 7 weeks (throughout gestation and lactation; 1 male and 1 female from each litter was treated themselves from PND 21-28)	Response relative to control Dose (mg/kg-d) / 0 / 64 / 160 / 400 / 1000 **Maternal effects** Body weight gain GD 0-20 0 / −3 / −4 / 0 / −16* Body weight gain PND 1-21 0 / +3 / −10 / +3 / +100* **F1 Reproductive effects** 0 / 64 / 160 / 400 / 1000 Viability index 96.4% / 98.7% / 98.2% / 99.4% / 74.1%* Lactation index 100% / 100% / 100% / 99.2% / 98.8% Sex ratio (% males) 54.4 / 52.3 / 50.9 / 53.4 / 52.1 Pup weight/litter PND 1 response relative to control 0 / +6 / +4 / +7 / −10 Pup weight PND 28 response relative to control M: 0 / +2 / 0 / 0 / −12* F: 0 / 0 / −4 / −2 / −8

Table 2-5. Evidence pertaining to developmental effects in animals following exposure to *tert*-butanol (continued)

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Inhalation</td></tr>
<tr><td>Nelson et al. (1989)

Sprague-Dawley rat; 15 pregnant dams/treatment
Whole body inhalation
Analytical concentration: 0, 2,200, 3,510, 5,030 ppm (0, 6,669, 10,640, 15,248 mg/m^3), 7 hr/d

GD 1–19</td><td>Maternal: Unsteady gait (no statistical tests reported), dose-dependent ↓ in body weight gain (results presented in figure only), dose-dependent ↓ in food consumption ranging from 7–36% depending on dose and time

Fetal

Percent change compared to control:

<table>
<tr><th>Dose (mg/m^3)</th><th>Number of live fetuses/litter</th><th>Resorptions per litter</th></tr>
<tr><td>0</td><td>0</td><td>0</td></tr>
<tr><td>6,669</td><td>0</td><td>+9</td></tr>
<tr><td>10,640</td><td>+15</td><td>−18</td></tr>
<tr><td>15,248</td><td>+8</td><td>0</td></tr>
</table>
Percent change compared to control:
<table>
<tr><th>Dose (mg/m^3)</th><th>Fetal weight (males)</th><th>Fetal weight (females)</th></tr>
<tr><td>0</td><td>0</td><td>0</td></tr>
<tr><td>6,669</td><td>−9*</td><td>−9*</td></tr>
<tr><td>10,640</td><td>−12*</td><td>−13*</td></tr>
<tr><td>15,248</td><td>−32*</td><td>−31*</td></tr>
</table>
Incidence:
<table>
<tr><th>Skeletal variation by litter</th><th>Skeletal variation by fetus</th></tr>
<tr><td>10/15</td><td>18/96</td></tr>
<tr><td>14/17</td><td>35/104</td></tr>
<tr><td>14/14</td><td>53/103*</td></tr>
<tr><td>12/12</td><td>76/83*</td></tr>
</table>
Skeletal variation by litter refers to the number of variations observed in the number of litters examined. Skeletal variation by fetus refers to the number of variations observed in the total number of fetuses examined. Fetuses are not categorized by litter.</td></tr>
</table>

* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from diet concentrations to mg/kg-d performed by study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

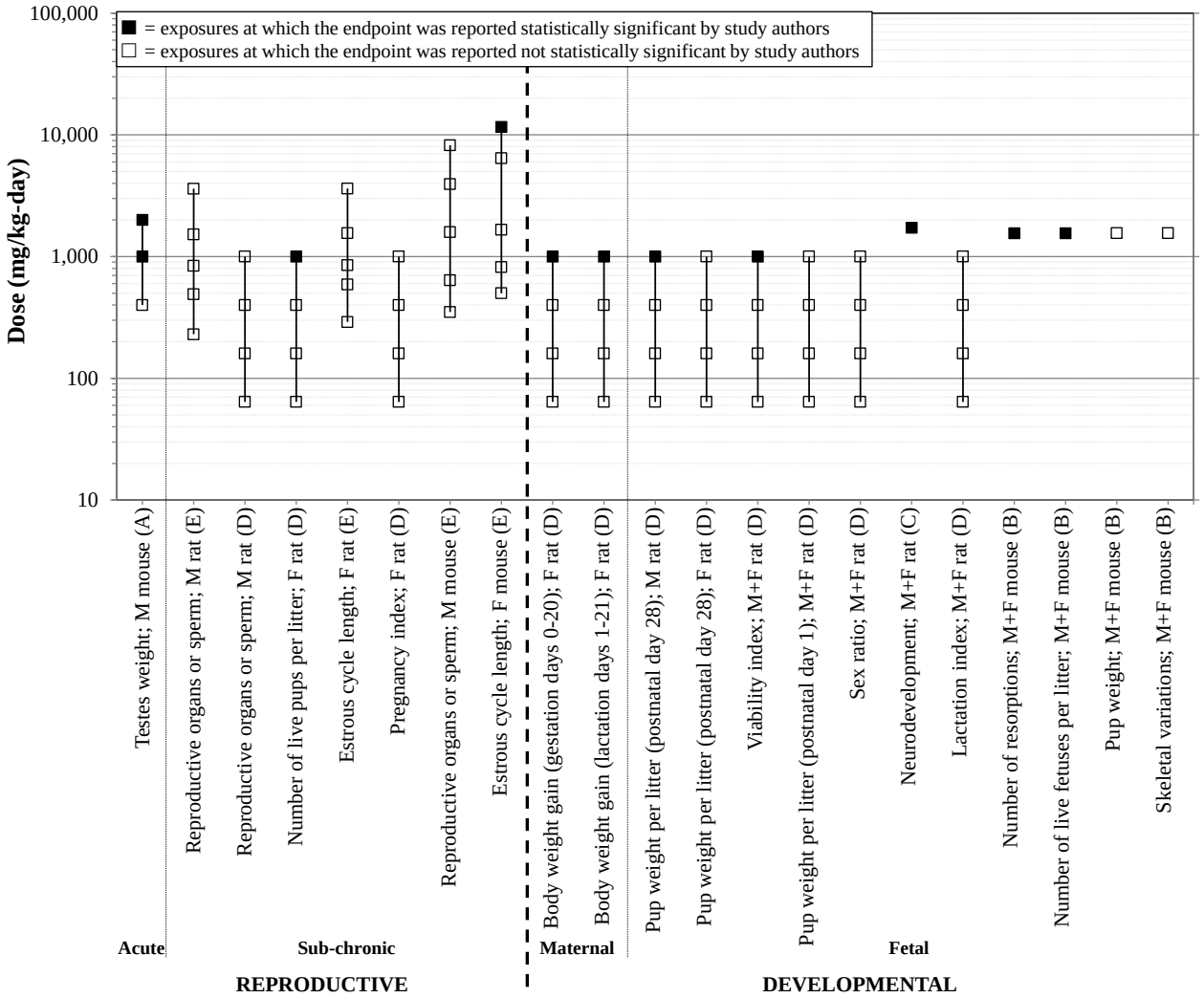
Table 2-6. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Oral	
Daniel and Evans (1982) Liquid diet (0, 0.5, 0.75, or 1.0%, w/v); GD6–20; Swiss Webster (Cox) mouse; 15 pregnant dams/treatment; after birth half the pups were nursed with their treated dams and the other half were fostered by untreated dams who recently gave birth 0 (isocaloric amounts of maltose/dextrin), 3,324, 4,879, or 6,677 mg/kg-d	Results were presented in figures only with no comparison to control or in tables with only comparison between pups fostered with their maternal dams and those fostered with untreated dams with no indication of dose. Results indicate • a dose-dependent increase in the time it took for the righting reflex with more time needed in animals maintained with maternal dams • a dose-dependent decrease in open field behavior with less activity with pups maintained with maternal dams • a dose-dependent decrease in rotorod performance with the pups from maternal dams having lower performances • a dose-dependent decrease in the amount of time the pups were able to avoid a cliff with animals maintained with their maternal dams having less avoidance time
Grant and Samson (1982) Surgical cannulation; male and female Long-Evans rat fetus; 8 control and 12 treated PND 4–7 (a period of brain growth) with consecutive daily doses of 1,440, 2,160, 600, and 2,690 mg/kg-d *tert*-butanol on PND 4, 5, 6, and 7, respectively (average daily dose of 1,723 mg/kg-d)	↓ Absolute and relative brain weights *Response relative to control (only relative data provided, but no changes were noted in body weight)*: 0, −16%* Brain biochemical parameters included: ↓ DNA levels in the hindbrain (−16%*) and ↓ protein levels in the forebrain (−15%*).
Inhalation	
Nelson et al. (1991) Whole body inhalation; Sprague-Dawley rat; 15 pregnant dams/treatment Analytical concentration: 0, 6,000, or 12,000 mg/m^3; 7 hr/d GD 1–19	Data were not presented specifically by dose nor were any tables or figures of the data provided Maternal toxicity was noted by decreased food consumption and body weight gains Results in offspring • increase in rotorod performance in high-dose group (16 versus 26 revolutions/min for controls and 3,500 mg/m^3 animals, respectively) • decreased time held on wire in the performance ascent test in the low-dose group (16 sec versus 10 sec for controls and 1,750 mg/m^3 animals, respectively) There were some differences in neurochemical measurements in the brain between control and treated offspring, but they were not related to dose

Table 2-6. Evidence pertaining to neurodevelopmental effects in animals following exposure to *tert*-butanol (continued)

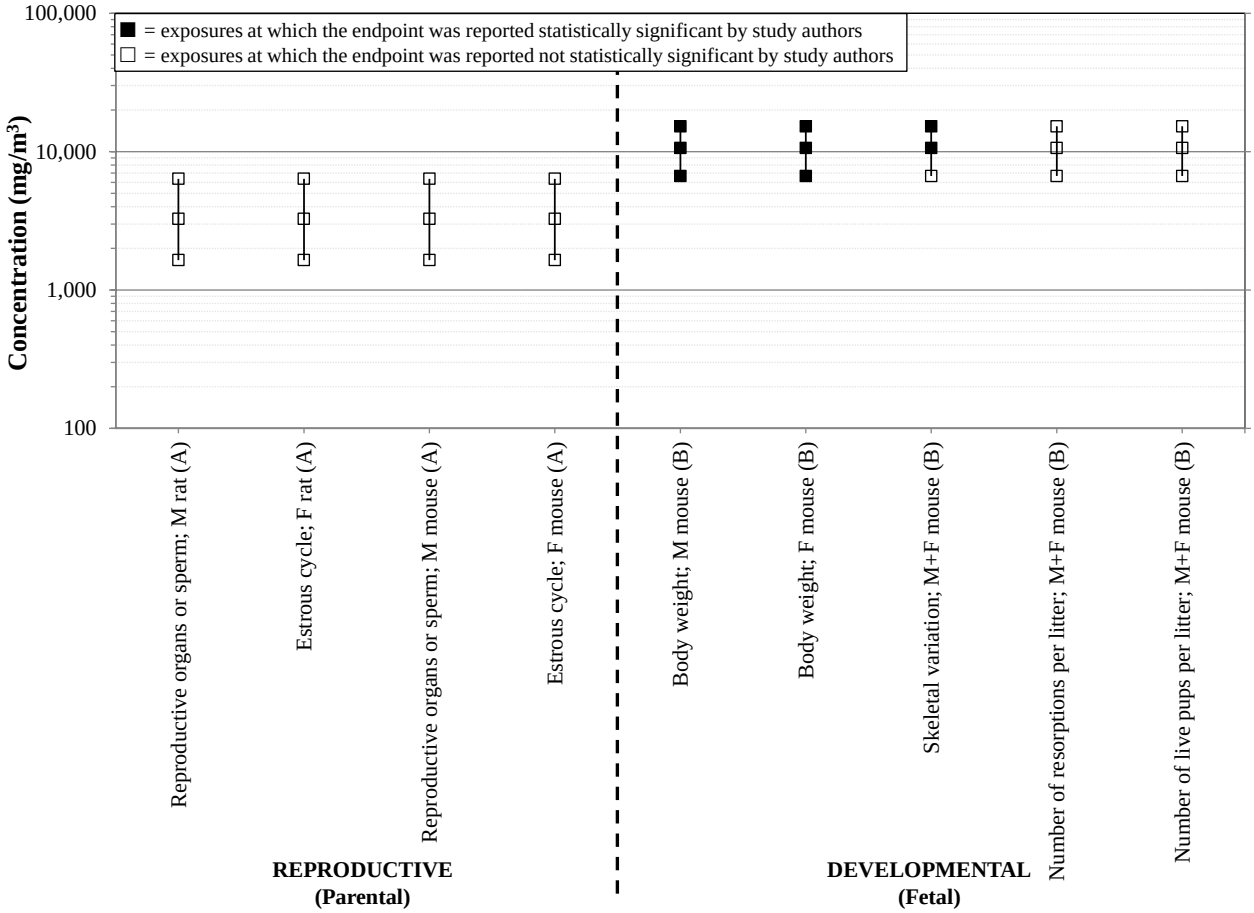
Reference and study design	Results
Nelson et al. (1991) Whole body inhalation; adult male Sprague-Dawley rats (18/treatment) mated to untreated females Analytical concentration: 0, 6,000, or 12,000 mg/m^3; 7 hr/d for 6 wk	Data were not presented specifically by dose nor were any tables or figures of the data provided Results (generally only specified as paternally treated versus controls) in offspring indicate • increase in rotorod performance (16 versus 20 revolutions/min for controls and 3,500 mg/m^3 animals, respectively) • decreased time in open field (less time to reach the outer circle of the field, 210 sec versus 115 seconds for controls and 3,500 mg/m^3 animals, respectively) There were some differences in neurochemical measurements in the brain between control and treated offspring, but they were not related to dose

* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from diet concentrations to mg/kg-d performed by study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.



Sources: (A) Billitti et al. (2005); (B) Faulkner et al. (1989); (C) Grant and Samson (1982); (D) Lyondell Chemical Company (2004); (E) NTP (1995)

Figure 2-4. Exposure-response array of reproductive, developmental and neurodevelopmental effects following oral exposure to *tert*-butanol.



Sources: (A) NTP (1997); (B) Nelson et al. (1989); (C) Nelson et al. (1991)

Figure 2-5. Exposure-response array of reproductive and developmental effects following inhalation exposure to *tert*-butanol.

2.5. Central Nervous System (CNS) Effects

Table 2-7. Evidence pertaining to CNS effects in animals following exposure to *tert*-butanol

Reference and study design	Results
Oral	
Grant and Samson (1981) Male Long-Evans rat; 4–6/treatment; daily intakes were estimated to be 700 to 2,800 mg/kg depending on the treatment and time Drinking water Scheduled-induced condition (maintained at 80% body weight): Treatment 1: 0.5% for 5 days, then 1% for 60 days Treatment 2:Drinking water stepwise increase with 0.25%, 0.50%, 1.0%, 2.0%, and 2.5% for 5 days each and 3.0%, and 3.5% for 10 days each, then 3.0% for 40 days Treatment 3: 1.0% for 5 days, 2.0% for 5 days, then 1.0% for 5 days and 3.0% for 90 days Home cage condition: Treatment 1: 80% body weight administered 0.5% 5 days, then 1.0% for 90 days Treatment 2: free access to food, 1.0% for 5 days, 2.0% for 10 days, and 3.0% for 90 days Treatment 3: 80% body weight on water After treatment, all animals were given water to replace t-butanol	• 5 of 15 animals treated with 3% *t*-butanol had to be removed from the experiment based on self-withdrawal and/or self-mutilation (2 died), trend of increased *t*-butanol intake and decreased food intake prior to removal. Symptoms were not observed with 1% *t*-butanol or with control. • 1 of 4 animals receiving 3.5% *t*-butanol self-mutilated, ceased drinking, and was removed from the experiment. • 60 days of 3% *t*-butanol was related to moderate seizure activity (withdrawal scores of 9, 5, and 6). • 90 days of 3% *t*-butanol had withdrawal scores of 12, 6, and 7 • At least one animal from every group had a withdrawal score of 0, and only animals exposed to 3% *t*-butanol had withdrawal scores of 6 or higher. Significantly higher withdrawal scores were observed in animals receiving greater amounts of *t*-butanol (Fischer's exact $p<0.002$). • Schedule-induced conditioning resulted in greater withdrawal symptoms over the home cage conditions. • Withdrawal symptoms were considered less than those observed with ethanol.

Table 2-7. Evidence pertaining to CNS effects in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results
Wood and Laverty (1979) Male Wistar rat; 12 rats/20-day treatment and 24 rats on time-course withdrawal sequence (4 rats withdrawn on days 4, 6, 8, 10, 12, and 14) Liquid diet; 44–54 mmol/kg (mean 47 mmol/kg); daily consumption was variable with spontaneous periods of abstinence in a few rats; blood alcohol levels 2–25 mmol/L 4–20 days	• Signs of intoxication in the 20-day study included docility and slight ataxia while on the diet. • One rat out of 12 in the 20-day study spontaneously withdrew from the diet on Day 10, and had signs of irritability, hyperactivity, and muscular rigidity. • Following removal of *t*-butanol after 20 days, animals initially appeared intoxicated. Head bobbing and paw shaking were observed 3–4 hours after withdrawal. Muscular rigidity, tail signs, abnormal gait, tremor, and irritability were apparent 5–6 hours after withdrawal. • 4/12 treated rats had spontaneous forelimb convulsions • 5/12 treated rats had audiogenic convulsions; resulted in 3 deaths. • In the surviving 8/12 rats, irritability, hyperreactivity, and muscular rigidity were considerable at 24 hours post-withdrawaland in some animals lasted for up to 72 hours. • In the time-course group, withdrawal symptoms were seen with as little as 4 days of *t*-butanol intake. Severity rating increased with increasing days on the diet.
Snell and Harris (1980) Male DBA/2J mouse; 20 mice/treatment 1.25% *t*-butanol via liquid diet 3,390 mg/kg-day (average) 7 days	• Shock avoidance behavior was impaired after cessation of treatment. • Latencies for experimental group was significantly ($p < 0.05$) longer than the controls. • One day after withdrawal, the number of trials where animals avoided shock was significantly ($p < 0.05$) greater in the control group (control: 35/150; *t*-butanol: 6/150). • Even 2 days after withdrawal, treated animals showed a significant ($p < 0.01$) deficit in avoidance response measured by both latency and number of avoidances (control: 51/100; *t*-butanol: 35/100). • At time of withdrawal, there was no difference in body temperature and withdrawal scores were low (0.20 ± 0.13, not significantly different from the control). **Body temperature** Control — *t*-butanol 3 hours after withdrawal — 37.6 ± 0.1 — 34.9 ± 0.4* 8 hours after withdrawal — 37.4 ± 0.1 — 35.7 ± 0.3* **Withdrawal scores** Control — *t*-butanol 3 hours after withdrawal — 0 — 0.30 ± 0.21 8 hours after withdrawal — 0 — 0.30 ± 0.15

Table 2-7. Evidence pertaining to CNS effects in animals following exposure to *tert*-butanol (continued)

Reference and study design	Results
Thurman et al. (1980) Female Sprague-Dawley rat; 12–14/treatment Gavage; 5.7% in saline every 8 hours for up to 6 days	14 symptoms of withdrawal were scored according to severity on a 1–3 point scale (every 2 hours beginning 6-8 hours after last treatment. • Treatment caused a steady increase in withdrawal score (results presented in a figure)
Inhalation	
McComb and Goldstein (1979a) Male Swiss-Webster mouse; 14 mice/treatment Two 3-day cycles separated by 24 hours of no treatment (withdrawal reaction observed for 24 hours following each exposure period); mice were maintained on either a low (range 3.5–6.4 mM) or high (range 6.5–10.0 mM) blood alcohol level	Withdrawal reaction consisted of signs of CNS hyperexcitability, including tremors, spontaneous convulsions, and convulsions elicited by handling **Withdrawal peak height** Low blood alcohol / High blood alcohol 3 days of exposure (n=11): 0.79 ± 0.12 / 1.43 ±0.07 6 days of exposure (n=13): 1.49 ± 0.08 / 1.63 ±0.10
McComb and Goldstein (1979b) Male Swiss-Webster mouse; 24 mice/treatment Mice received an initial i.p. priming dose of 6.8 or 10.1 mmol/kg *t*-butanol; *t*-butanol vapor concentration was maintained between 50 and 80 µmol/m^3 for 24 hours then were steadily increased to maintain a constant blood alcohol level Withdrawal was measured after 1, 3, 6 or 9 days	• Withdrawal reaction consisted of signs of CNS hyperexcitability, including tremors, spontaneous convulsions, and convulsions elicited by handling. • Intensity of withdrawal symptoms was related to blood levels and duration of exposure (results provided in figure only). o At mean blood levels of 5mM t-butanol (range 2.4-6.6 mM), severity of withdrawal reaction rose steadily through day 9. o At mean blood levels of 8.5 mM (range 6.8-10.7 mM) and above, a ceiling of withdrawal levels occurred at a height of approximately 1.5. • Peak withdrawal reactions occurred 3–5 hours after removal of *t*-butanol and was 4-5 times greater than with ethanol.

* Statistically significant $p \leq 0.05$ as determined by study authors.

2.6. Other Systemic Effects (Body Weight, Liver, and Urinary Bladder)

Table 2-8. Evidence pertaining to effects on body weight in animals following oral exposure to *tert*-butanol

Reference and study design	Results
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	Body weight in treated animals lower than controls by ~7% ($p < 0.05$); (results only provided in a figure)
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 64 / −2 / 64 / 0 160 / −4 / 160 / −2 400 / +2 / 400 / +1 1,000 / −7 / 1,000 / +4
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 230 / −4 / 290 / +2 490 / −5* / 590 / +1 840 / −12* / 850 / +1 1,520 / −17* / 1,560 / −2 3,610 / All dead / 3,620 / −21*
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 350 / −1 / 500 / +3 640 / +1 / 820 / −1 1,590 / −4 / 1,660 / +4 3,940 / −14* / 6,430 / −6 8,210 / −24* / 11,620 / −15* High-dose females had a significantly lower initial weight, but also had a significantly lower body weight gain indicating that there was some effect of treatment

Table 2-8. Evidence pertaining to effects on body weight in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, 10 mg/mL) M: 0, 90, 200, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 90 / −15 / 180 / −2 200 / −18 / 330 / −5 420 / −24 / 650 / −21 Only animals that survived at the end of 2 years were evaluated for body weight. Note: statistical significance not determined by study authors.
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, 20 mg/mL); M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Body weight / Dose (mg/kg-d) / Body weight 0 / 0 / 0 / 0 540 / +1 / 510 / −2 1,040 / −2 / 1,020 / −3 2,070 / −1 / 2,110 / −12 Only animals that survived at the end of 2 years were evaluated for body weight. Note: statistical significance not determined by study authors.

[a]There was a significant decrease in survival in the high-dose group.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Table 2-9. Evidence pertaining to liver effects in animals following oral exposure to *tert*-butanol

Reference and study design	Results
Liver weight	
Acharya et al. (1995) Wistar rat; 5–6 males/treatment Drinking water (0 or 0.5%), 0 or 575 mg/kg-d 10 weeks	No significant treatment-related effects (results were only provided in a figure)
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 64 / −1 / 0 160 / −3 / +1 400 / −2 / −1 1,000 / +8 / +16* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 64 / −4 / −4 160 / −7 / −5 400 / +2 / +1 1,000 / +8 / +3
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 230 / −2 / +4 490 / +1 / +8* 840 / +5 / +20* 1,520 / +8 / +31* 3,610 / All dead / All dead **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 290 / +11* / +9* 590 / +10* / +9* 850 / +12* / +11* 1,560 / +15* / +16* 3,620 / +9* / +41*
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Percent change compared to control: **Males**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 350 / +2 / +3 640 / −1 / −2 1,590 / −1 / +5 3,940 / 0 / +14* 8,210 / −16 / +22* **Females**: Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 500 / −1 / −4 820 / −5 / −3 1,660 / −8 / −9* 6,430 / −2 / +6 11,620 / −6 / +13*

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Table 2-9. Evidence pertaining to liver effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5 or 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	Percent change compared to control: **Males** / **Females** Dose (mg/kg-d) / Absolute weight / Relative weight / Dose (mg/kg-d) / Absolute weight / Relative weight 0 / 0 / 0 / 0 / 0 / 0 90 / +2 / +7 / 180 / −14* / −8 200 / +8 / +11 / 330 / −3 / −1 420 / +1 / +14* / 650 / −6 / +9* Only animals sacrificed at 15 months were evaluated for organ weights.
Histopathology	
Acharya et al. (1997; 1995) Wistar rat; 5–6 males/treatment Drinking water (0, 0.5%), 0, 575 mg/kg-d 10 weeks	↑ liver glycogen (~ 7 fold)* ↑incidence of centrilobular necrosis, vacuolation of hepatocytes, loss of hepatocyte architecture, peripheral proliferation, and lymphocyte infiltration (incidences and results of statistical tests not reported)
Lyondell Chemical Company (2004) Sprague-Dawley rat; 12/sex/treatment Gavage 0, 64, 160, 400, or 1,000 mg/kg-d At least 9 weeks beginning 4 weeks prior to mating through mating (up to 2 weeks), gestation, and lactation.	No treatment-related effects observed.
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, or 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	No treatment-related effects observed (histopathology data for the 13-week study were not provided)
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	No treatment-related effects observed (histopathology data for the 13-week study were not provided)

Table 2-9. Evidence pertaining to liver effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results			
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, 10 mg/mL) M: 0, 90, 200, or 420[a] mg/kg-d F: 0, 180, 330, or 650[a] mg/kg-d 2 years	No treatment-related effects observed.			
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, 20 mg/mL) M: 0, 540, 1,040, or 2,070[a] mg/kg-d F: 0, 510, 1,020, or 2,110 mg/kg-d 2 years	**Males**		**Females**	
	Dose (mg/kg-d)	Incidence of fatty change	Dose (mg/kg-d)	Incidence of fatty change
	0	12/59	0	11/60
	540	5/60	510	8/60
	1,040	8/59	1,020	8/60
	2,070	29/59*	2,110	6/60

[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

Table 2-10. Evidence pertaining to liver effects in animals following inhalation exposure to *tert*-butanol

Reference and study design	Results				
Liver Weight					
NTP (1997) F344/N rat; 10/sex/treatment Analytical concentration: 0, 135, 270, 540, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m^3) 6 hr/d, 5 d/wk 13 weeks	Percent change compared to control:				
		Males		**Females**	
	Dose (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	0	0	0	0
	406	−8	−8	0	+3
	824	−2	−1	0	0
	1,643	+1	−1	+3	+2
	3,273	+10	+7	+9	+9*
	6,368	+5	+5	+4	+8*
NTP (1997) B6C3F1 mouse; 10/sex/treatment Analytical concentration: 0, 134, 272, 542, 1,080, or 2,101 ppm (0, 406, 824, 1,643, 3,273 or 6,368 mg/m3) 6 hr/d, 5 d/wk 13 weeks	Percent change compared to control:				
		Males		**Females**	
	Dose (mg/m^3)	Absolute weight	Relative weight	Absolute weight	Relative weight
	0	0	0	0	0
	406	−1	0	+1	−4
	824	+4	+9	+1	+5
	1,643	+7	+5	+5	+1
	3,273	−8	-2	+2	+9*
	6,368	+5	+7	+8	+21*

* Statistically significant $p \leq 0.05$ as determined by the study authors.
Conversion from ppm to mg/m^3 is 1 ppm = 3.031 mg/m^3.
Percentage change compared to control = (treated value – control value) ÷ control value × 100.

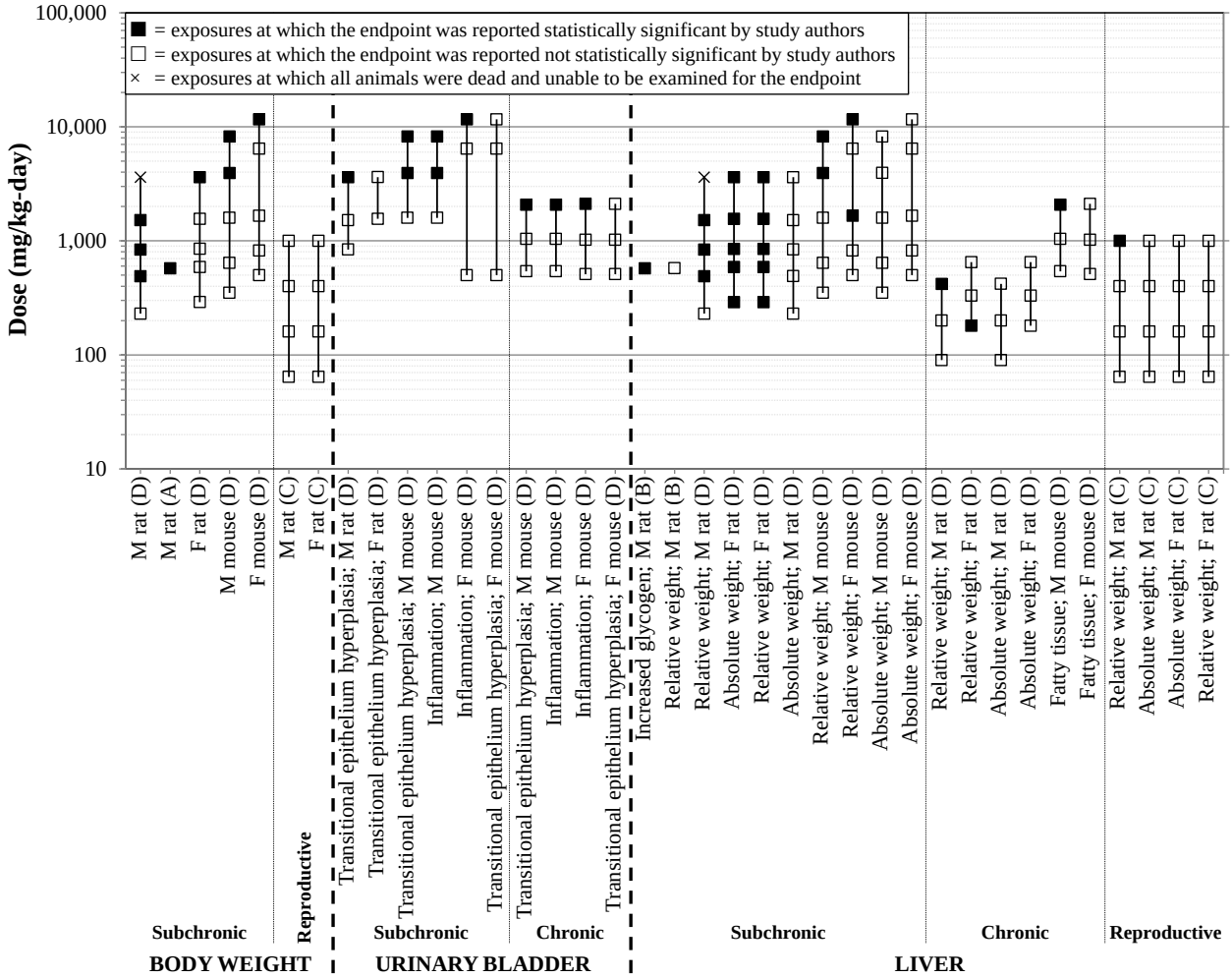
Table 2-11. Evidence pertaining to urinary bladder effects in animals following oral exposure to *tert*-butanol

Reference and study design	Results
Histopathology	
NTP (1995) F344/N rat; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 230, 490, 840, 1,520, 3,610[a] mg/kg-d F: 0, 290, 590, 850, 1,560, 3,620[a] mg/kg-d 13 weeks	Incidence (severity): **Males** / **Females** Dose (mg/kg-d) — Transitional epithelial hyperplasia 0 — 0/10 230 — not evaluated 490 — not evaluated 840 — 0/10 1,520 — 1/10 (3.0) 3,610 — 7/10* (2.9) Dose (mg/kg-d) — Transitional epithelial hyperplasia 0 — 0/10 290 — not evaluated 590 — not evaluated 850 — not evaluated 1,560 — 0/10 3,620 — 3/10 (2.0) Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
NTP (1995) B6C3F$_1$ mouse; 10/sex/treatment Drinking water (0, 2.5, 5, 10, 20, 40 mg/mL) M: 0, 350, 640, 1,590, 3,940, 8,210[a] mg/kg-d F: 0, 500, 820, 1,660, 6,430, 11,620[a] mg/kg-d 13 weeks	Incidence (severity): **Males** / **Females** Dose (mg/kg-d) — Transitional epithelial hyperplasia — Inflammation 0 — 0/10 — 0/10 350 — not evaluated 640 — not evaluated 1,590 — 0/10 — 0/10 3,940 — 6/10* (1.3) — 6/10* (1.3) 8,210 — 10/10* (2.0) — 10/10* (2.3) Dose (mg/kg-d) — Transitional epithelial hyperplasia — Inflammation 0 — 0/10 — 0/10 500 — 0/1 — 0/1 820 — not evaluated 1,660 — not evaluated 6,430 — 0/10 — 0/10 11,620 — 3/9 (2.0) — 6/9* (1.2) Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
NTP (1995) F344/N rat; 60/sex/treatment (10/sex/treatment evaluated at 15 months) Drinking water (0, 1.25, 2.5, 5, or 10 mg/mL) M: 0, 90, 200, 420[a] mg/kg-d F: 0, 180, 330, 650[a] mg/kg-d 2 years	No treatment-related effects observed

Table 2-11. Evidence pertaining to urinary bladder effects in animals following oral exposure to *tert*-butanol (continued)

Reference and study design	Results					
NTP (1995) B6C3F$_1$ mouse; 60/sex/treatment Drinking water (0, 5, 10, or 20 mg/mL) M: 0, 540, 1,040, 2,070[a] mg/kg-d F: 0, 510, 1,020, 2,110 mg/kg-d 2 years	Incidence (severity):					
	Males			**Females**		
	Dose (mg/kg-d)	Transitional epithelial hyperplasia	Inflam-mation	Dose (mg/kg-d)	Transitional epithelial hyperplasia	Inflam-mation
	0	1/59 (2.0)	0/59	0	0/59	0/59
	540	3/59 (1.7)	3/59 (1.7)	510	0/60	0/60
	1,040	1/58 (1.0)	1/58 (1.0)	1,020	0/59	0/59
	2,070	17/59* (1.8)	37/59* (2.0)	2,110	3/57 (1.0)	4/57* (2.0)
	Severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked					

[a]The high-dose group had an increase in mortality.
* Statistically significant $p \leq 0.05$ as determined by study authors.
Conversions from drinking water concentrations to mg/kg-d performed by study authors.



Sources: (A) Acharya et al. (1995); (B) Acharya et al. (1997; 1995); (C) Lyondell (2004); (D) NTP (1995)

Figure 2-6. Exposure-response array of body weight, liver effects, and urinary bladder effects following oral exposure to *tert*-butanol.

2.7. Genotoxic Effects

Table 2-12. Evidence pertaining to genotoxic effects of *tert*-butanol in vitro and in vivo studies

Species/ cell line	Test system (strain/species)	Exposure (dose/ concentration)	Results Metabolic activation		Reference
			–S9	+S9	
In vitro					
Bacterial systems					
Salmonella typhimurium	Reverse mutation (TA98, TA100, TA1535, TA1537, TA1538)	10 mg/plate	–	–	Zeiger et al. (1987)
		2.9–10,000 µg/plate	–	–	ARCO (1994e)
	Reverse mutation (TA102)	1–4 mg/plate	ND	+	Williams-Hill et al. (1999)
	Reverse mutation (TA98, TA100, TA102, TA1535, TA1537)	5–5,000 µg/plate	–	–	McGregor et al. (2005)
E.coli	Reverse mutation (WP2 uvrA/PKM101)	5–5,000 µg/plate	–	–	McGregor et al. (2005)
Neurospora crassa	Reverse mutation, ad-3A locus (allele 38701)	1.75mol/L	–	–	Dickey et al. (1949)
Saccharomyces cerevisiae	Mitochondrial mutation (K5-5A, MMY1, D517-4B and DS8)	4.0% (vol/vol)	+[a]	ND	Jimenez et al. (1988)
Mammalian cells – rodent					
Mouse lymphoma cells L5178Y $TK^{+/-}$	Gene mutation	625–5,000 mg/mL	–	–	McGregor et al. (1988)
		2.4–32 µL/mL	–	–	ARCO (1994a)
Chinese hamster ovary	Sister-chromatid exchange	1.25–5 mg/mL	–	–	NTP (1995)
		0.31–20 µL/mL (-S9); 0. 625–20 µL/mL (+S9)	–[b]	–	ARCO (1994b)
	Chromosomal aberrations	1.25–5 mg/mL	–	–	NTP (1995)
Rat fibroblasts	DNA damage (comet assay)	0.44 mmol/L (IC_{50})	+[c]	ND	Sgambato et al. (2009)

Species/ cell line	Test system (strain/species)	Exposure (dose/ concentration)	Results Metabolic activation		Reference
			–S9	+S9	
Mammalian cells – human					
Human HL-60 leukemia cells	DNA damage (comet assay)	1–30 mmol/L	+	ND	Tang et al. (1997)
In vivo					
Mammalian – rodent					
Male Kunming mouse liver, kidney and lung cells	DNA adducts	0.1–1,000 µg/kg b.w.	+	NA	Yuan et al. (2007)
B6C3F$_1$ mouse peripheral blood cells	Micronucleus formation	2.5–40 mg/mL drinking water	–	NA	NTP (1995)

ND = not determined; NA = not applicable

[a]Effect is predicted to be due to mitochondrial membrane composition.

[b]Results were stated to be statistically increased in the 20 µg/mL with and without activation and the 10 µg/mL with activation, but results did not meet positive criteria for the assay.

[c]DNA damage was completely reversed with increase in time of exposure.