OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Tertiary-Butyl Acetate

Cancer Inhalation Unit Risk Factor

Technical Support Document for Cancer Potency Factors
Appendix B

August 2018

Air and Site Assessment and Climate Indicator Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

Page Intentionally Left Blank

Tertiary-Butyl Acetate

Cancer Inhalation Unit Risk Factor

Technical Support Document for Cancer Potency Factors
Appendix B

Prepared by the

Office of Environmental Health Hazard Assessment (OEHHA)

Lauren Zeise, Ph.D., Director

Authors

John D. Budroe, Ph.D.

Rona M. Silva, Ph.D.

Kathleen Vork, Ph.D.

Technical Reviewers

David Siegel, Ph.D.

August 2018

Table of Contents

List of Acronyms

8-OHdG	8-hydroxydeoxyguanosine
$^{13/14}C$	Carbon-13 or -14 labeled
AIC	Akaike Information Criterion
ARB	Air Resources Board
AST	Aspartate Aminotransferase
BMD	Benchmark Dose
$BMDL_{05}$	BMD 95% Lower Confidence Limit
BMDS	Benchmark Dose Software
BR	Breathing Rate
BrdU	5-bromo-2'-deoxyuridine
BROD	7-benzoxyresorufin-O-debenzylase
BW	Body Weight
CAR	Constitutive Androstane Receptor
CAS	Chemical Abstracts Service
CE	Carboxylesterase
CHO	Chinese Hamster Ovary
CNS	Central Nervous System
CPN	Chronic Progressive Nephropathy
CSF	Cancer Slope Factor
CTI	California Toxics Inventory
CV	Unit Conversion Value
CYP	Cytochrome P-450
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EROD	7-ethoxyresorufin-O-deethylase
ETBE	Ethyl *tert*-Butyl Ether
FA	Fractional Absorption
GD	Gestation Day
HBA	2-Hydroxyisobutyrate
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
ILO	International Labour Organization
IUR	Inhalation Unit Risk Factor
K_{ow}	Octanol-water Partition Coefficient
LC_{50}	Lethal Concentration that causes death in 50% of the population
LDH	Lactate Dehydrogenase
LED_{10}	the lower 95% confidence bound on the dose associated with a 10% increased risk of cancer
LMWPF	Low-Molecular-Weight Protein Fraction
LOAEL	Lowest Observed Adverse Effect Level
MC	Metabolic Conversion Factor
MPD	2-methyl-1,2-propanediol
MTBE	Methyl *tert*-Butyl Ether
MTD	Maximum Tolerated Dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MV	Minute Volume
MWR	Molar Weight Ratio
NA	Not Applicable
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
NT	Not Tested
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
PB	Phenobarbital
PCNA	Proliferating Cell Nuclear Antigen
PE	Post Exposure
PND	Post-natal Day
PROD	7-pentoxyresorufin-O-dealkylase
SCE	Sister Chromatid Exchanges
SDS	Sodium Dodecyl Sulfate
SEM	Standard Error of the Mean
SRP	Scientific Review Panel
T_3	Triiodothyronine
T_4	Thyroxine
TBA	*Tertiary*-butanol
TBAc	*Tertiary*-butyl acetate
TEH	Transitional Epithelium Hyperplasia
TMP	2,2,4-trimethylpentane
TSH	Thyroid-Stimulating Hormone
U1	2–hydroxymethyl-isopropyl acetate
U2	2-hydroxyisobutyric acid
U4	TBA-glucuronide
U6	2 hydroxymethyl-isopropyl acetate-glucuronide conjugate
U8	*tertiary*-Butyl-2-hydroxyl-acetate glucuronide
US EPA	United States Environmental Protection Agency
WB	Whole Body

Introduction

This document explains the evaluation of carcinogenicity and derivation of a cancer inhalation unit risk factor (IUR) for *tertiary*-butyl acetate (TBAc; Chemical Abstracts Service (CAS) No: 540-88-5). Cancer IURs are used to estimate lifetime cancer risks associated with inhalation exposure to a carcinogen.

Basis for Cancer Hazard Identification and IUR Development

The National Toxicology Program (NTP) conducted a long-term carcinogenicity bioassay of *tertiary*-butanol (TBA) in rats and mice (NTP, 1995). The study provides evidence for the carcinogenicity of TBA in rats and mice. Cancer slope factors (CSFs) can be developed from a modeled dose-response analysis of the TBA rat and mouse tumor data. TBA is a primary metabolite of TBAc in rodents (Groth and Freundt, 1994; Cruzan and Kirkpatrick, 2006). It is also a presumed primary metabolite in humans based upon rodent data for TBAc, and human and rodent data for chemical surrogates of TBAc (Hong *et al.*, 1997; Bernauer *et al*., 1998; Nihlén *et al.*1998; Amberg *et al.*, 1999; Nihlén *et al.*, 1999; Amberg *et al.*, 2000; 2001). Thus, TBAc should also be considered a carcinogen. A CSF and cancer IUR for TBAc has been calculated for use in the Hot Spots program from the TBA CSF using an appropriate metabolic conversion factor and route-to-route extrapolation.

Document Peer Review and Adoption Mechanism

OEHHA is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2). In implementing this requirement, OEHHA develops cancer IURs for carcinogenic air pollutants listed under the Air Toxics Hot Spots program. The TBAc IUR was developed using the most recent “Air Toxics Hot Spots Program Technical Support Document for Cancer Potency Factors”, finalized by OEHHA in 2009. The TBAc cancer IUR draft document was made available for public review on August 14, 2015, on the OEHHA website at http://www.oehha.ca.gov and was revised in response to public comments. The draft document received external peer review by the Scientific Review Panel on Toxic Air Contaminants (SRP) on December 14, 2016. This November 2017 version of the draft document has been revised in response to SRP comments. Once finalized, the document will be adopted by the Director of OEHHA for use in the Air Toxics Hot Spots program. After adoption, the document will be included in Appendix B of the Air Toxics Hot Spots Program Technical Support Document for Cancer Potency Factors.

Uses and Outdoor Emissions

TBAc is commonly used as a solvent in a variety of products including industrial coatings, inks, adhesives, industrial cleaners and degreasers.

TBAc is listed as a substance for which emissions must be quantified under the Air Toxics Hot Spots Program. According to the California Air Resources Board (ARB), facilities listed under the Hot Spots program as emitting TBAc in the 2012 – 2015

database years include pipe painting and coating companies, aerospace manufacturing plants, and military bases (ARB, 2017).

The California Toxics Inventory (CTI) provides emissions estimates for stationary (point and aggregated point), area-wide, on-road mobile (gasoline and diesel), off-road mobile (gasoline, diesel, and other), and natural sources. Stationary sources include point sources provided by facility operators and/or air districts pursuant to the Air Toxics Hot Spots Program, and aggregated point sources estimated by the ARB and/or air districts. Area-wide sources are those that do not have specific locations and are spread out over large areas such as consumer products and unpaved roads. The 2010 draft CTI summary table lists state area-wide TBAc emissions of 27.1 tons/year (ARB, 2010).

Residential and Worker Exposures

The expected routes of exposure to TBAc are inhalation, dermal contact, and ingestion (hand-to-mouth). Published data regarding specific work- and home-site investigations into actual worker and residential exposure measurements, respectively, are not available. However, exposures may occur by working in or living in close proximity to facilities that produce, use, or emit TBAc.

Using dispersion modeling software, ARB, the South Coast Air Quality Management District, and the San Joaquin Valley Unified Air Pollution Control District produced modeled TBAc exposure estimates for various scenarios (Bus *et al.* 2015). Under these scenarios (Table 1, below), acute near-facility and occupational exposure levels were estimated at concentrations of 3920 μg/m^3 (825 ppb) and 1280 μg/m^3 (269 ppb), respectively. Modeled chronic near-facility levels ranged from concentrations of 1.4 – 63.9 μg/m^3 (0.29 – 13.5 ppb), and modeled occupational exposure levels were to concentrations as high as 532,000 μg/m^3 (112,000 ppb). Occupational exposure levels modeled by ARB (2006) did not consider the use of personal protective equipment (*e.g*. facemasks) which could limit worker exposure to TBAc. ARB (2006) described their occupational estimates as “realistic worst case” indoor exposures. Assumptions for their modeled chronic 24-hour continuous concentrations (Scenarios 15-17) are similarly likely to be health-protective, as they do not account for time away from the TBAc source. Exposure during non-working hours was assumed to be the same as the modeled near-facility concentration of 19.7 μg/m^3 (4.15 ppb; ARB, 2006). None of the modeled exposure scenarios noted the inclusion of oral exposures. The information shown in Table 1 is for informational purposes only; it does not affect OEHHA’s calculations of the IUR or CSF for this compound.

Table 1. Modeled acute and chronic *tertiary*-butyl acetate (TBAc) exposure estimates associated with various exposure scenarios (Adapted from Bus *et al*., 2015).

Acute Exposure Scenarios	Scenario	TBAc Air Concentrations		24-hr Continuous Concentrations	
		µg/m^3	ppb	µg/m^3	ppb
Near Facility[†]: Repainting a 1 million-gallon water tank (volume source)	1	3920	825	NC	NC
Occupational: Maximum 1-hr concentration at auto refinish spray booth	2	1280	269*	53[a]	12.5*[a]
Consumer: Aerosol paint use in living room, peak concentration[b]	3	2.19 x 10^6	4.61 × 10^5*	NC	NC
Chronic Exposure Scenarios					
Southern California environmental exposure[c]	4	2.8	0.59	NC	NC
Near Facility[†]: Average of 5 auto refinish facilities with spray booths and stacks (point sources)[d]	5	1.4	0.29	NC	NC
Repainting at a refinery	6	1.6	0.35	NC	NC
Repainting at a sewage treatment facility	7	3.1	0.66	NC	NC
Largest general automotive repair shop (max annual average)[e]	8	10.1	2.13*	NC	NC
RJ auto refinish facility with capped stack (horizontal dispersion)	9	13.5	2.84	NC	NC
Hypothetical large auto refinish facility (max annual average)	10	19.7	4.15	NC	NC
Central California auto refinish facility without a spray booth (volume source)[f]	11	57.5	12.1	NC	NC
Hypothetical large auto refinish facility	12	63.9	13.5	NC	NC
Occupational: Repainting at a refinery	13	6.88	1.45	2.3	0.5
Repainting at a sewage treatment facility	14	13.2	2.78	4.4	0.9
Large brake shop facility[g]	15	14,000	2960	3348	705
Small brake shop facility[g]	16	35,000	7400	8348	1757
Personal brake shop workspace[g]	17	532,000	112,000	126,682	26,666

Scenarios were modeled by the California Air Resources Board (ARB; Scenarios 2, 4, 8, 10, and 15-17), South Coast Air Quality Management District (Scenarios 1, 6, 7, and 12-14), San Joaquin Valley Air Pollution Control District (Scenarios 5, 9, 11), or Lyondell Chemicals

Company (Scenario 3) using regional meteorological data. OEHHA was only able to confirm modeled concentrations attributed to ARB (2006).
Abbreviation: NC – Not calculated.
[†]In the table by Bus *et al.* (2015) and the publication by ARB (2006), the term, "near-source" was used instead of "near-facility" as shown in the table above. The associated concentrations were estimated at the facility fence-line or 20-30 meters from the point source unless otherwise noted.
*Conversion value differs from Bus *et al.* (2015).
[a]It is unclear how these values were calculated.
[b]Room size adjusted to garage size (125 m^3) reflecting likely use area (Bus *et al.*, 2015).
[c]Estimates for this category do not include indoor exposures (ARB, 2006).
[d]Suggested by Bus *et al.* (2015) to be more typical than the chronic near-source scenarios with the highest exposures.
[e]Does not include potential ambient background TBAc levels (ARB, 2006).
[f]Estimated concentration is 37 meters downwind from this source (Bus *et al.*, 2015).
[g]According to ARB (2006),the 24-hour time-weighted concentrations for these modeled scenarios combine 8-hour workplace and near-source exposure levels to estimate exposures of automotive workers who may also live near a TBAc source (*e.g.* brake shop facility). The near-source level of 19.7 µg/m^3 from Scenario 10 was used to represent non-work exposure concentrations. Average 24-hour exposure = 5/7 work days per week [(8/24 work hours per day) × (Work exposure µg/m^3) + (16/24 non-work hours per day) x (Non-work exposure µg/m^3)] + 2/7 non-work days per week x (non-work exposure). This ARB equation was used by OEHHA to calculate the 24-hour continuous brake shop concentrations shown above, which differ from those reported by Bus *et al.* (2015).

Non-cancer Health Effects of TBAc

This section provides a general synopsis of the non-cancer health effects that have been associated with exposure to TBAc.

Odor and Sensory Detection of TBAc in Acute Exposure Studies of Humans

Acute human exposure experiments by Cain and Schmidt (2009) showed that the strong, camphor-like odor of TBAc (Bus *et al.* 2015) was detected with 50% accuracy when the TBAc air concentration was approximately 8 ppb (38 µg/m^3). When compared to modeled exposures, 8 ppb (38 µg/m^3) fell between calculated chronic environmental exposures in Southern California (2.8 µg/m^3; 0.6 ppb; ARB, 2006) and estimated concentrations near the fence-line of a hypothetical large auto refinishing facility (63.9 µg/m^3; 13.45 ppb; Bus *et al.*, 2015). A TBAc "odor threshold" of 71 ppb (340 µg/m^3) was reported in humans by Nagata (2003), but it is unclear what this threshold means with respect to accuracy of detection.

Irritation of the eyes and/or nose has been reported in humans acutely exposed at TBAc concentrations between 47 and 200 ppm (223 and 950 mg/m^3; Cain and Schmidt, 2009; HSDB, 2017), one or two orders of magnitude above that required for 50% olfactory detection. When compared to modeled exposures, the TBAc concentration of 200 ppm (950 mg/m^3) was close to the acute consumer exposure to 461 ppm (2190 mg/m^3) calculated for use of aerosol paint in a garage-size (125 m^3) living room (Bus *et al.*, 2015; Table 1).

Responses such as extreme eye/nose irritation, headaches, and/or weakness have been reported in humans at concentrations ranging from 950 to 3300 ppm TBAc (4513 - 14,252 mg/m^3) by the Hazardous Substances Data Bank (HSDB, 2017). This information references a peer-reviewed Encyclopedia of Occupational Health and Safety published by the International Labour Organization (ILO) of the United Nations (original not available). According to the HSDB (2017), the ILO review stated that although the principal symptoms in cases of occupational exposure are headaches and irritation of the mucous membranes of the nose and eyes, other symptoms include vertigo, palpitations, gastrointestinal disorders, anemia, cutaneous lesions, dermatitis, and affections of the liver. It is unclear which exposure conditions are necessary to produce these secondary effects; to our knowledge, there are no published case reports or data on specific work-site investigations into occupational TBAc exposures. Acute TBAc exposure studies in humans are summarized in Table 2, below.

Table 2. **Summary of acute *tertiary*-butyl acetate exposure studies in humans.**

Reference	Study population and Exposure Method	Results	Point of Departure[a]
Nagata (2003)	Subjects, age 20-50 yrs (n = 6). Exposure period unknown.	71 ppb (0.34 mg/m^3) odor threshold	Not stated
Cain and Schmidt (2009)	Males and females, age ≈ 22 yrs (n = 29). Nose-only inhalation for 16 min (2 sec/sniff, ≥480 sniffs).	8 ppb: 50% detection via odor	Not stated
	Males and females, age ≈ 22 (n = 26). Ocular exposure for 15 min/eye (180 trials, 10 sec/trial).	177 ppm: 50% detection via eye irritation	NOAEL = 47 ppm (estimated)
HSDB (2017)	Subjects' age and number unknown. Inhalation exposure for unknown time-period.	200 - 300 ppm (950 – 1425 mg/m^3): slight irritation of the eyes and nose. 3300 ppm (14,252 mg/m^3): extreme irritation of the nose and eyes, headaches and weakness.	Not stated

Abbreviations: 50% detection – level at which individuals were able to detect correctly the odor of TBAc in half of the trials. NOAEL – No Observable Adverse Effect Level
[a]Derived by the original authors, not OEHHA.

Dermal Irritation and Sensitization Potential of TBAc in Rabbits and Guinea Pigs

According to reviews by the German Committee for Occupational Exposure Limits (MAK Commission, 2016) and the European Chemicals Agency (ECHA, 2017a), undiluted TBAc has been dermally tested on white rabbits at 866 mg/mL for four

hours and 2000 mg/kg for 24 hours. This application produced minimal skin irritation and no systemic toxicity, swelling, or evidence of tissue destruction. Authors of the second reviewed study concluded that the single dermal exposure at 2000 mg/kg was insufficient to produce systemic toxicity (ECHA, 2017a). A separate study performed in guinea pigs showed that TBAc did not cause dermal sensitization (ECHA, 2017b). OEHHA found no respiratory sensitization studies.

Effects of Acute or Subacute TBAc Exposure in Rodents

Acute Inhalation Exposure to TBAc

OEHHA identified two peer-reviewed studies (Cruzan and Kirkpatrick, 2006; Yang *et al.*, 2010) and one industry study (Stillmeadow, 1997) describing rodent responses to acute inhaled TBAc exposure. These studies were conducted using TBAc exposures to concentrations ranging from 470 – 5066 ppm (2223 – 24,067 mg/m^3) for up to six hours.

Results showed that rats and mice exhibited transient decreases in body weight (BW), difficulty breathing, impaired coordination, central nervous system (CNS) effects (*e.g.* tremors, rapid head shaking), and/or early mortality when acutely exposed to TBAc from 1873 – 3000 ppm (8898 – 14,252 mg/m^3; Cruzan and Kirkpatrick, 2006; Yang *et al.*, 2010). A No Observed Adverse Effect Level (NOAEL) of 470 ppm (2223 mg/m^3) was reported by Stillmeadow, Inc. (1997) for their study in which rats were monitored for mortality and clinical signs of toxicity. A Lowest Observed Adverse Effect Level (LOAEL) of 3000 ppm (14,252 mg/m^3) was reported by Cruzan and Kirkpatrick (2006) in mice observed with prostration (physical weakness), hypoactivity, and ataxia (loss of muscle coordination). Summaries of the acute TBAc inhalation exposure studies in rodents reviewed by OEHHA are shown in Table 3, below.

Table 3. Summary of acute *tertiary*-butyl acetate (TBAc) inhalation studies in rodents.

Reference	Animal Model & TBAc Exposure	Results Relative to Controls	Point of Departure[a]
Cruzan and Kirkpatrick (2006)	Male and female rats (age 7-8 wks; n = 5/sex/group). Nose-only inhalation of 0, 1873, 3502, or 5066 ppm (0, 8898, 16,637, or 24,067 mg/m^3) for 6 hrs	3502 and 5066 ppm: 80% female mortality during exposure, and 100% male and female mortality within 1 hr PE, respectively. Congested lungs in early decedents. Other effects: transient exaggerated breathing, immobility and lethargy (3502 ppm), rapid head shaking (1873 ppm), and staggering and tremors (at unstated concentrations) that resolved by 24 hrs PE.	LOAEL = 1873 ppm for transient CNS effects LC_{50} = 4200 ppm for males and females combined
	Male mice (age 7-8 wks; n = 5/group). Inhalation (WB) of 0 or 3000 ppm (0 or 14,252 mg/m^3) for 6 hrs	3000 ppm: Prostration during exposure. Hypoactivity, ataxia, and dyspnea 40 min PE.	LOAEL[b] = 3000 ppm for behavioral and respiratory effects
Yang *et al.* (2010)	Male rats (age 8 wks; n = 6/group/timepoint). Nose-only inhalation of 0, 503, or 2001 ppm (0, 2387, or 9506 mg/m^3) for 4 hrs. Necropsy 2, 7, or 15 days PE.	2001 ppm: Transient ↓ in BW gains and ↑ in AST on Day 2 PE only. Liver necrosis on Day 2 and onward (1 rat/day; two rats total. It is unclear whether the second rat came from the PE Day 7 or 15 necropsy). 503 ppm: No significant (biological or statistical) differences from control	LC_{50} > 2000 ppm.
Stillmeadow (1997)	Male and female rats (unknown age, n = 5/sex/group). Inhalation of 0 or 470 ppm (0, or 2223 mg/m^3) for 4 hrs	No deaths or clinical signs	NOAEL[b] = 470 ppm (2223 mg/m^3)

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; AST – serum Aspartate Aminotransferase, a liver enzyme; BW – Body weight; CNS – Central Nervous System; LC_{50} – Concentration that causes mortality in 50% of the test population; LOAEL – Lowest Observable Adverse Effect

Level; NOAEL – No Observable Adverse Effect Level; PE – Post Exposure; WB – Whole Body.
[a]Derived by the original authors unless otherwise noted.
[b]According to review by OEHHA.

Subacute Inhalation Exposure to TBAc

Comprehensive subacute TBAc exposure experiments (duration 1-14 days) in rodents were reported by Cruzan and Kirkpatrick (2006). Their results suggest that sensitive endpoints include but are not limited to liver effects (*e.g.*, centrilobular hepatocyte hypertrophy and increased liver weights) at ≥375 ppm (903 mg/m^3), and CNS effects (*e.g.*, drooling and impaired coordination/ambulation) at ≥1501 ppm (7131 mg/m^3). Although statistical comparisons by sex and species were not reported, some results suggest that sexually dimorphic patterns of liver sensitivity may exist as female mice and male rats appear more sensitive to TBAc-induced hepatocyte hypertrophy and/or increased liver weights than their opposite-sex counterparts. The subacute TBAc inhalation experiments by Cruzan and Kirkpatrick (2006) are summarized in Table 4.

Table 4. Summary of subacute *tertiary*-butyl acetate (TBAc) inhalation studies in rodents.

Reference	Animal Model & TBAc Exposure	Results Relative to Controls	Point of Departure[a]
Cruzan and Kirkpatrick (2006)	Male and female mice (age 7-8 wks; n = 5/sex/group). Inhalation (WB) of 0, 190, 375, 751, or 1501 ppm (0, 903, 1782, 3568, or 7131 mg/m^3) 6 hr/day for 14 days	≥ 375 ppm: minimal centrilobular hepatocyte hypertrophy in 20%, 40%, and 100% females, respectively 751 and 1501 ppm: ↑ liver weights in females 1501 ppm: Impaired coordination. Minimal hepatocyte hypertrophy in 60% males	NOAEL = 190 ppm for female liver effects
	Male and female rats (age 7-8 wks; n = 5/sex/group) Nose-only inhalation of 0, 120, 430, or 1643 ppm (0, 570, 2043, or 7805 mg/m^3) for 2 wks (6 hr/day, 5 days/wk)	430 ppm: Minimal hepatocyte hypertrophy in 40% males. Renal hyaline droplet accumulation in 80% males (same at 1643 ppm). 1643 ppm: Transient unsteady gait immediately PE, with resolution by the next morning. In males, ↑ centrilobular hepatocyte hypertrophy and ↑ liver weights. In 20% females, non-significant hepatocyte hypertrophy.	NOAEL = 120 ppm for liver hypertrophy in males

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; NOAEL – No Observable Adverse Effect Level; PE – Post Exposure; WB – Whole Body.
[a]Derived by the original authors, not OEHHA.

Subacute Oral (Gavage) Exposure to TBAc and Reproductive/Developmental Effects

OEHHA found two studies of the reproductive and developmental effects of subacute TBAc exposures in rats (Yang *et al.*, 2007; Faber *et al.*, 2014) in rats. In both studies, oral TBAc doses (400 – 1600 mg/kg/day) were administered to pregnant rats for at least 14 days starting from gestation day (GD) 6, and dams were sacrificed prior to parturition. Exposures resulted in maternal CNS effects (*e.g*. drooling and impaired coordination/ambulation) at ≥ 400 mg/kg BW/day (Faber *et al.* 2014), and feto-embryo effects (*e.g*. supernumerary ribs and delayed ossification) at ≥800 mg/kg BW/day (Yang *et al.*, 2007). At 1600 mg/kg BW/day, adverse maternal clinical signs including piloerection, decreased locomotion, dragging body, hunched posture, fur loss, vocalization, reddish tears and vaginal discharge, nasal hemorrhage, coma,

congestion/hemorrhage of the duodenum (the first part of the small intestine), atrophy of the spleen, and early mortality were observed in addition to significantly ($p < 0.05$) decreased thymus and increased adrenal gland weights relative to controls (Yang *et al.,* 2007; Faber *et al.*, 2014).

Because Yang *et al.* (2007) did not observe adverse maternal effects at <1600 mg/kg BW/day, they concluded that the treatments were minimally embryotoxic at the non-maternally toxic dose of 800 mg/kg/day, and non-teratogenic at the maternally toxic dose of 1600 mg/kg/day. NOAELs of 800 and 400 mg/kg/day were assigned by the authors for dam maternal effects and feto-embryo developmental effects, respectively. Citing Johnson *et al.* (1987), they stated that agents with a developmental hazard index (maternal NOAEL/fetal NOAEL) less than 3 represent maternally-targeted agents. Thus, given their index of 2 (800/400 mg/kg/day), this type of evidence suggests that TBAc is maternally targeted in rats and not selectively toxic to the conceptus (Yang *et al.*, 2007). Like Yang *et al.* (2007), Faber *et al.* (2014) concluded that large, bolus, oral doses of TBAc are primarily maternotoxic since they observed no effect on fetal viability at any of the tested doses, or on fetal BW at 400 and 800 mg/kg/day. Studies by Yang, Faber, and their respective colleagues are summarized in Table 5, below.

Table 5. Summary of oral subacute *tertiary*-butyl acetate (TBAc) exposures and reproductive/developmental effects in rats.

Reference	Animal Model & TBAc Exposure	Results Relative to Controls	Point of Departure[a]
Yang *et al.* (2007)	Pregnant female rats (age 11 wks; n = 22/group). Oral gavage of 0, 400, 800, or 1600 mg/kg/day during GDs 6-19	400 and 800 mg/kg: ↑ incidence of dilated feto-embryo ureter. ≥800 mg/kg: ↑ feto-embryo supernumerary ribs. Delayed ossification in bones of/near the tail and/or palm. 1600 mg/kg: Maternal deaths, adverse clinical signs, transient ↓ food consumption and BW gains, ↑ adrenal gland and liver weights, and ↓ thymus weights. ↓ fetal male BW.	NOAEL = 400 mg/kg for feto-embryo toxicity. NOAEL = 800 mg/kg for maternal toxicity.
Faber *et al.*(2014), maternal and feto-embryo toxicity experiments	Pregnant female rats (age 12 wks; n = 22/group). Oral gavage of 0, 400, 800, 1000, or 1600 mg/kg/day during GDs 6-20	≥ 400 mg/kg: Tearing and drooling in dams. ↓ fetal BW. ≥ 800 mg/kg: transient ↓ in food consumption. Dose-related CNS effects on coordination and ambulation. 1600 mg/kg: ↓ thymus and ↑ adrenal gland weights. ↑ liver, kidney, and adrenal gland weights relative to brain weight. ↓ thymus relative to brain weight. Adverse clinical effects and early mortality.	LOAEL[b] = 400 mg/kg for maternal and feto-embryo toxicity

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BW – Body weight; CNS – Central Nervous System; GD – Gestation Day; NOAEL – No Observable Adverse Effect Level; PE – Post Exposure; WB – Whole Body.

[a]Derived by the original authors, not OEHHA.

[b]According to review by OEHHA.

Effects of Subchronic TBAc Exposure in Rodents

Subchronic Inhalation Exposure to TBAc

No chronic TBAc exposure studies were found. However, in addition to the subacute oral experiment summarized above, Faber *et al.* (2014) also reported the general toxicity effects of subchronic TBAc inhalation exposures in rats and mice. These experiments are discussed below.

The most sensitive endpoints reported by Faber *et al.* (2014) were 1) at ≥100 ppm (475 mg/m^3), increased male rat kidney weights relative to BW, with kidney lesions, hyaline droplets, and basophilic tubules that were, according to the authors, consistent with α2u-globulin nephropathy; and 2) at ≥400 ppm (1900 mg/m^3), transiently impaired equilibrium in mice during exposure. α2u nephropathy is characterized by the accumulation of α2u-globulin droplets in the proximal tubule of kidney nephrons, followed by single cell necrosis, formation of granular casts at the junction of the proximal tubule and the descending loop of Henle, and presence of regenerative tubules (Swenberg, 1993). No differences in α2u-globulin accumulation were observed by immunohistochemistry or enzyme-linked immunosorbent assay (ELISA) among the TBAc-exposed groups, but incidence and severity of tubular basophilia were slightly (non-significantly) higher in rats exposed to 1600 ppm (7601 mg/m^3).

Other effects included but were not limited to transient hyperactivity and excessive grooming in mice exposed to ≥100 ppm TBAc (475 mg/m^3), and increased liver, adrenal gland, and kidney weights in male and/or female rats at 1600 ppm TBAc (7601 mg/m^3). Because the occurrence of hyperactivity and excessive grooming was temporally inconsistent and only noted occasionally in mice exposed to 100 ppm (475 mg/m^3), Faber *et al*. (2014) did not consider the effects to be treatment-related at this concentration. Similar reasoning was given for the impaired equilibrium effects observed in mice exposed to 400 ppm (1900 mg/m^3). Consequently, the authors assigned NOAELs of 100 ppm (475 mg/m^3) for hyperactivity and excessive grooming, and 400 ppm (1900 mg/m^3) for impaired equilibrium. The subchronic inhalation experiments performed by Faber *et al.* (2014) are summarized in Table 6, below.

Table 6. Summary of Subchronic *tertiary*-butyl acetate (TBAc) inhalation exposures in rodents.

Reference	Animal Model & TBAc Exposure	Results Relative to Controls	Point of Departure[a]
Faber *et al*. (2014), general toxicity experiments	Rats (age 7 wks; n = 10/sex/group). Inhalation (WB) of 0, 100, 400, 1600 ppm (0, 475, 1900, or 7601 mg/m^3) for 13 wks (6 hr/day, 7 days/wk)	≥100 ppm: ↑ male kidney weights relative to BW, and kidney lesions, hyaline droplets, and basophilic tubules consistent with α2u-globulin nephropathy. 1600 ppm: Changes in levels of locomotor activity in males. Transient ↓ food consumption and BW gains in males and females. ↑ male/female liver weights, ↑ male adrenal weights, and ↑ female kidney weights.	NOAEL = 400 ppm for subchronic motor effects
	Mice as above	≥100 ppm: acute, transient hyperactivity and excessive grooming PE 100 and 1600 ppm: occasionally labored breathing during exposure 400 and 1600 ppm: occasionally impaired equilibrium during exposure 1600 ppm: ↑ male and female liver weights. In females, centrilobular hepatocellular hypertrophy (n = 1/10) and non-significantly increased PCNA labeling. In males, ↓ T_4	NOAEL = 100 ppm for hyperactivity and excessive grooming PE NOAEL = 400 ppm for impaired equilibrium during-exposure

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BW – Body weight; NOAEL – No Observable Adverse Effect Level; PCNA - Proliferating Cell Nuclear Antigen; PE – Post Exposure; T_4 – Thyroxine, a thyroid hormone.

[a]Derived by the original authors, not OEHHA.

Subchronic Inhalation Exposure to TBAc and Reproductive/Developmental Effects

In addition to the general toxicity experiments described above, Faber *et al.* (2014) also reported effects of a subchronic reproductive toxicity screening study in rats. Parental males and females were exposed to TBAc at concentrations ranging from 100 – 1600 ppm (475 – 7601 mg/m^3) for 6 hours/day, 7 days/week, for 10 weeks prior to mating, and throughout mating, gestation, and lactation periods until the day before sacrifice (109-110 days for males; 108-119 days for females). Female exposures were halted after GD 20 but resumed on postnatal day (PND) 5.

Pups were examined starting on PND 0; monitored for developmental effects; and euthanized on PND 24, or exposed post-weaning from PND 22-26 (n=1/sex/litter) to the same TBAc concentration as their parents and then euthanized on PND 27 for examination.

The only significant ($p < 0.05$) effect was transiently decreased BW in parental male rats exposed to 1600 ppm TBAc (7601 mg/m^3) versus control. A slight (non-significant) decrease in the rate of BW gains was reported for female pups exposed at the highest TBAc concentration, but no other effects were noted for pups. A summary of the subchronic TBAc exposure studies reviewed by OEHHA are shown in Table 7, below.

Table 7. Summary of subchronic *tertiary*-butyl acetate (TBAc) inhalation exposures and reproductive/developmental effects in rats.

Reference	Animal Model & TBAc Exposure	Results Relative to Controls	Point of Departure[a]
Faber *et al.* (2014), reproductive toxicity experiments	Parental rats (age 6 wks; n = 10/sex/group). Inhalation (WB) of 0, 100, 400, or 1600 ppm (0, 475, 1900, or 7601 mg/m^3) for 109-110 days in males, or 108-119 days in females, during pre-mating, mating, gestation, and lactation periods. Similar exposure concentrations in pups from PNDs 22-26.	No reproductive effects at any tested concentration. 1600 ppm: transiently ↓ BW from exposure days 14-56 in parental male rats. Non-significant decrease in rate of female pup BW gains.	NOAEL = 1600 ppm for clinical observations, survival, reproductive performance, gross- or histo-pathology, sperm parameters, implantation sites, gestation length and parturition

Abbreviations: ↑ – increase resulting in significant ($p \leq 0.05$) difference; ↓ – decrease resulting in significant ($p \leq 0.05$) difference; BW – Body weight; NOAEL – No Observable Adverse Effect Level; PND – Post Natal Day; WB – Whole Body.
[a]Derived by the original authors, not OEHHA.

Page Intentionally Left Blank

TERTIARY-BUTYL ACETATE (TBAc)

CAS No: 540-88-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 2017)

Molecular formula	$C_6H_{12}O_2$
Molecular weight	116.16
Description	colorless liquid
Density	0.8593 at 25°C /4°C
Boiling point	95.1°C
Vapor pressure	47 mm Hg @ 25°C
Solubility	Practically insoluble in water; miscible with common industrial organic solvents including ethanol, ethyl ether, acetic acid and chloroform.
Conversion factor	1 ppm = 4.74 mg/m^3
Uses	Solvent

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor	1.3×10^{-6} (µg/m^3)$^{-1}$
Slope Factor	4.7×10^{-3} (mg/kg-day)$^{-1}$
Oral Slope Factor	5.0×10^{-3} (mg/kg-day)$^{-1}$

[Male rat renal tubule tumor data from *tert*-butanol drinking water exposure study (NTP, 1995), Multistage Cancer model (US EPA, 2017).]

The derivation of these values is based on the male F344 rat kidney tumor incidence data for *tertiary*-butanol (TBA), the primary rodent metabolite of TBAc (NTP, 1995). The TBA exposure to rats was by the oral (gavage) route. The TBA cancer risk estimate was calculated using benchmark dose (BMD) methodology. The lower 95 percent confidence limit on the BMD level associated with a 5 percent tumor response ($BMDL_{05}$) was derived by the multistage cancer model in Benchmark Dose Software (BMDS) version 2.6, and used as the point of departure. Linear extrapolation from the $BMDL_{05}$ to the origin was used to determine the slope of the dose-response curve for low level exposure, which is the cancer slope factor (CSF). The oral and inhalation CSFs describe the excess cancer risk from an oral or inhalation exposure, respectively, to 1 mg/kg-day TBAc over a 70-year lifetime. The oral CSF for TBAc was derived from this value assuming 71% metabolism of TBAc to TBA and a molar weight ratio (MWR) of 0.64. The TBAc cancer inhalation unit risk factor (IUR) describes the excess cancer risk incurred due to an inhalation exposure to 1 µg/m^3 TBAc over a 70-year lifetime. The IUR and inhalation CSF assumes that 95% of an inhaled dose of TBAc is absorbed systemically. Data from Cruzan and Kirkpatrick (2006) were used to derive the fraction of TBAc metabolized to TBA and the TBAc inhalation absorption value.

III. CARCINOGENICITY

No carcinogenicity studies are available for TBAc. However, exposure to TBA, the presumptive primary human metabolite of TBAc, has been demonstrated to cause tumors in rats and mice. There are no human carcinogenicity data for either compound.

Metabolism and Pharmacokinetics

Little information is available describing the absorption, distribution, metabolism, or excretion of TBAc in humans. There are currently no published physiologically-based pharmacokinetic models for TBAc. However, insights regarding potential human metabolism and toxicokinetics of TBAc can be obtained from rodent studies of TBAc and/or TBA metabolism, and chemicals with a *tert*-butyl functional group similar to TBAc.

TBAc has been shown in rats to be absorbed following both inhalation and oral exposure. Once absorbed, TBAc is distributed to all major tissues. TBAc does not appear to accumulate extensively in body tissues, and is either exhaled as the parent compound or metabolized. One of the primary rodent metabolites of TBAc is TBA. Inhalation exposures of TBAc to rats can result in blood concentrations of TBA that approach or exceed blood concentrations of TBAc, depending on dose. Both TBAc and TBA are eliminated by exhalation in rats but the unchanged compounds do not appear appreciably in urine. TBA metabolites are eliminated in rat urine, but elimination in rat feces is negligible.

Rat metabolism of TBAc to TBA has been shown to take place by both carboxylesterase (CE)- and cytochrome P450 (CYP)-dependent pathways. Human and mouse data for CE-mediated metabolism of TBAc to TBA are not available, but both human and mouse CEs have a wide substrate specificity and are distributed within a wide variety of tissues, suggesting that this metabolic pathway could be operative in both humans and mice. Data on the biological activity of methyl *tertiary*-butyl ether (MTBE) and ethyl *tertiary*-butyl ether (ETBE) can be useful in the consideration of TBAc and TBA carcinogenicity. Both MTBE and ETBE have been shown to induce tumors in rodents. MTBE induces tumors in rats: leukemias, lymphomas and Leydig cell testicular tumors after oral exposure, and renal tubular and Leydig cell testicular tumors after inhalation exposure. OEHHA (1999b) has developed an IUR for MTBE. ETBE induces liver tumors in rats after inhalation exposure.

Like TBAc, MTBE and ETBE are each metabolized to TBA by similar CYP enzymes, and are somewhat similar in structure, making them potential surrogates for comparing TBAc metabolism between rats and humans. MTBE and ETBE pharmacokinetic (PK) data are available for both rats and humans. Both compounds are metabolized *in vivo* by CYPs to TBA, aldehydes and acids in both rats and humans. Studies indicate that the metabolism and pharmacokinetics of MTBE and

ETBE and the TBA produced from metabolism of those compounds are reasonably similar in these two species. Less corresponding information was located for mice.

MTBE and TBA both appear in the exhaled breath of humans after both oral intake and inhalation exposure in a similar manner, suggesting that MTBE and possibly TBAc lack significant first-pass metabolism in humans. First-pass metabolism can greatly reduce the concentration of an ingested chemical before it reaches the systemic circulation. The MTBE and ETBE data described above suggest that the metabolism of TBAc to TBA seen in rats can also occur in humans. The studies that provided the data used to formulate these conclusions are described below.

Absorption of TBAc and Blood Accumulation of TBA in Rats

TBAc is readily absorbed into the circulation of rats following oral and inhalation exposures, and TBA, a primary metabolite of TBAc, accumulates in the blood (Groth and Freundt 1994; Cruzan and Kirkpatrick, 2006). Two separate experiments on the uptake and accumulation of inhaled TBAc were conducted in Sprague-Dawley rats (Groth and Freundt 1994). The first experiment involved a single continuous 5-hour (300-minute) exposure to approximately 440 ppm TBAc (2090 mg/m^3) via tracheal cannula, and concurrent, frequent blood measurements to track the time course of TBAc and TBA accumulation in blood. Immediate and continuously increasing blood concentrations of TBAc were observed for approximately 3.3 hrs (168 minutes) into the exposure. For the remaining exposure period, the blood concentration plateaued suggesting steady state was reached (Figure 1). In contrast, blood concentrations of TBA rose at a steady rate throughout the entire exposure period and peaked at approximately 350 µM, at the end of the exposure (300 minute time-point; Figure 1). At that time, blood TBA levels were slightly higher than those for TBAc (approximately 350 versus 280 µmol/L blood; Figure 1).

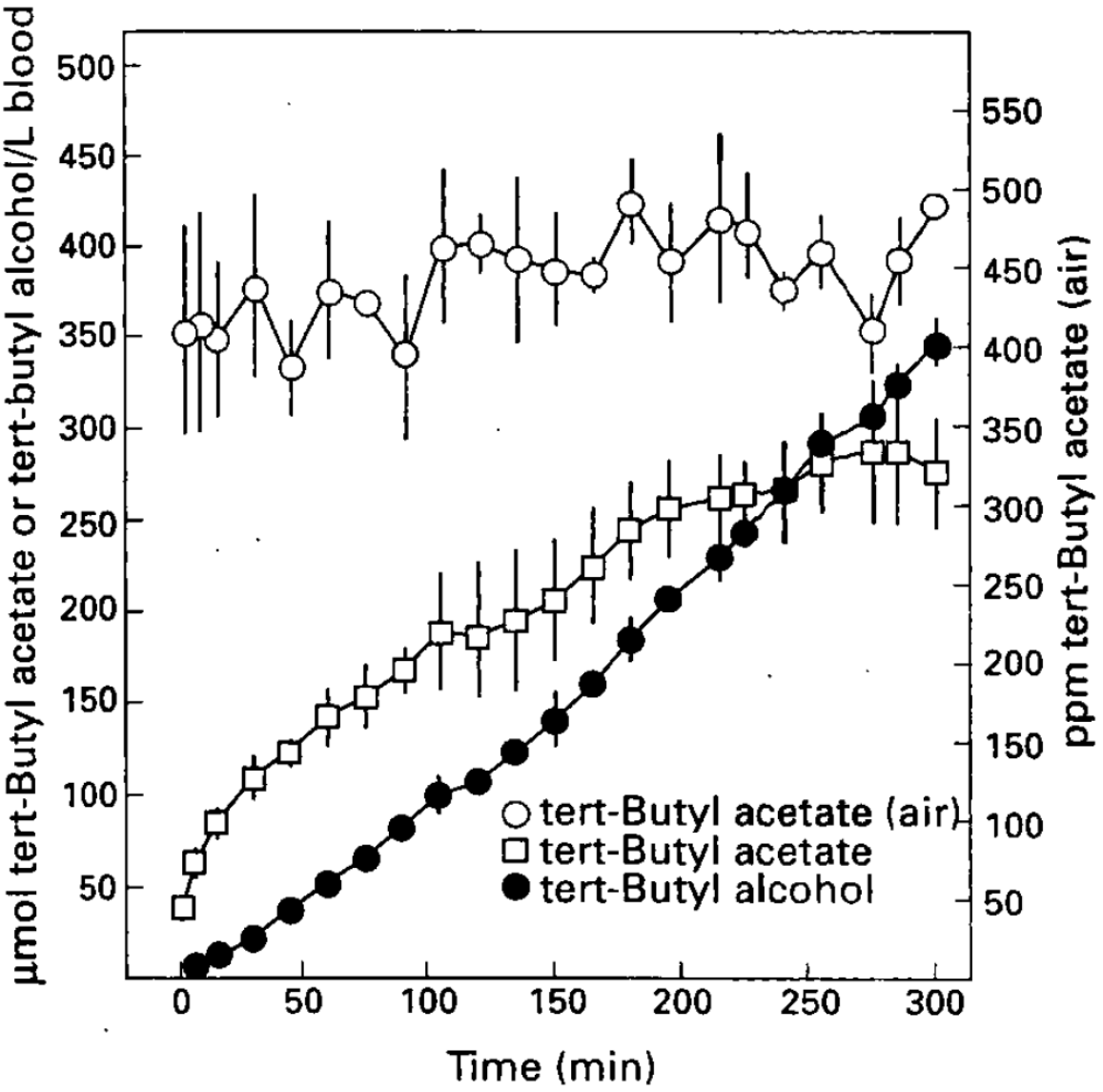

Figure 1. Concentrations of *tertiary*-butyl acetate (TBAc) and *tertiary*-butanol in blood of five rats, and concentrations of TBAc in air of five experiments during a continuous 5-hour exposure to TBAc. Each data point represents the mean concentration from the five experiments ± standard error of the mean (SEM), for a specific time-point. The mean ± SEM of all concentrations of TBAc measured in air was 436.6 ± 29.9 ppm (2074 ± 142 mg/m^3; Groth and Freund, 1994; Figure 1).

In the second experiment by Groth and Freundt (1994), rats inhaled a single continuous air concentration of about 900 ppm TBAc (4276 mg/m^3) over a period of 4.25 hours (255 minutes). Blood was collected concurrently and 0.75 hours (45 minutes) after exposure. TBAc and TBA blood concentrations rose in parallel, at steady rates throughout the exposure period indicating similar accumulation kinetics in blood during that time (Figure 2). Peak blood concentrations reached approximately 540 and 470 µmol/L, respectively (Figure 2). Once TBAc exposures

ceased, TBAc blood concentrations immediately dropped, decreasing by approximately 50% within the 45-minute observation period, while TBA blood levels appeared to remain steady during the continued metabolism of TBAc to TBA (Figure 2).

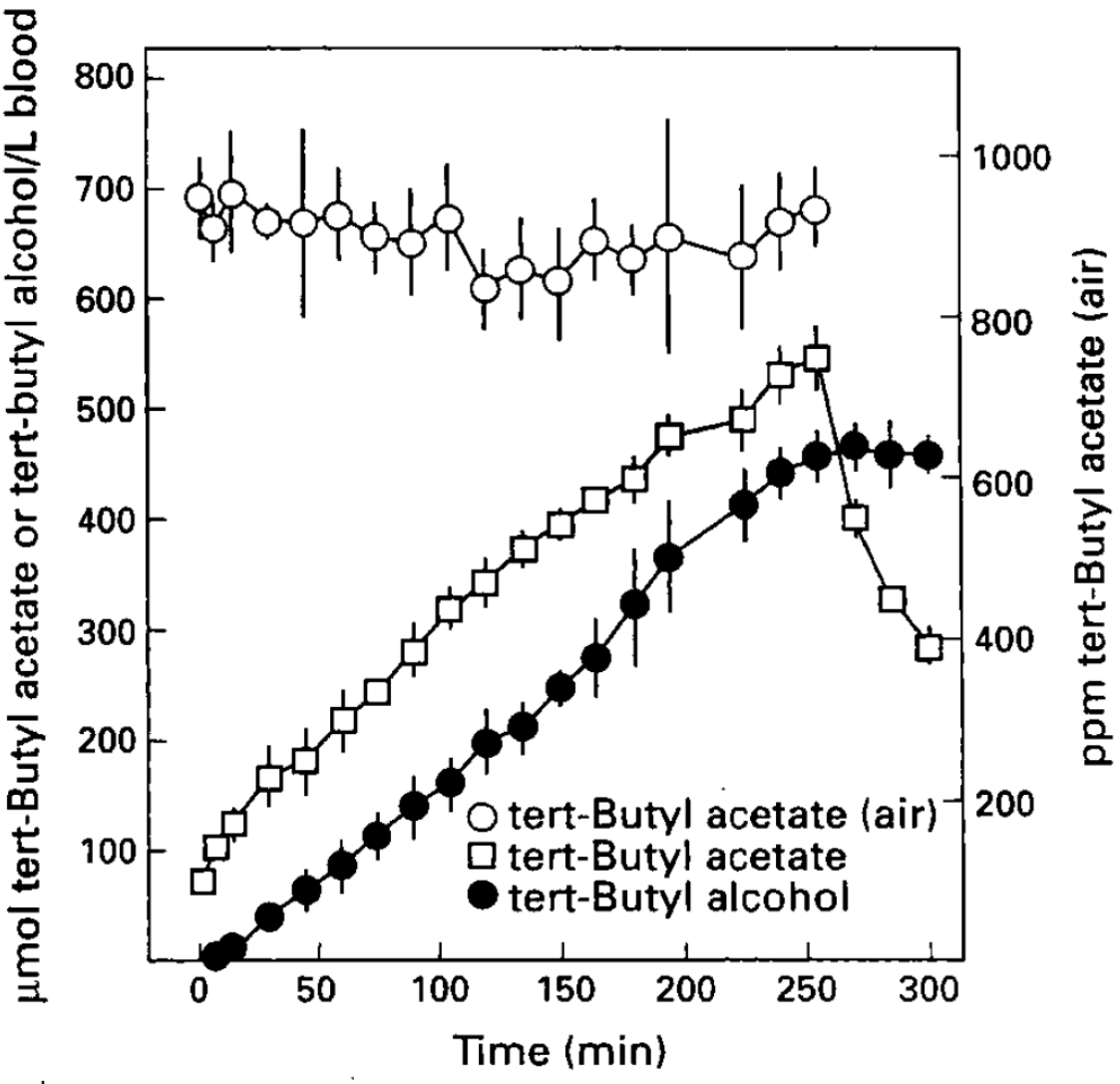

Figure 2. Concentrations of *tertiary*-butyl acetate (TBAc) and *tertiary*-butanol in blood of five rats, and concentrations of TBAc in air of five experiments during and after a continuous 255-min exposure to TBAc. Each data point represents the mean concentration from the five experiments ± SEM, for a specific time-point. The mean ± SEM of all concentrations of TBAc measured in air was 898.4 ± 77.8 ppm (4268 ± 370 mg/m^3; Groth and Freund, 1994; Figure 2).

An abstract of a limited study that tracked the elimination kinetics of TBAc in both human and rat blood provides some insight about the half-life of TBAc in these two species. TBAc was introduced at an unstated concentration into the blood of male humans and female Sprague-Dawley rats, and elimination times were compared (Essig *et al.*, 1989.) Blood half-lives of 300 and 270 minutes were reported in rats

and humans, respectively. However, OEHHA notes that results from these preliminary studies do not generate the same degree of confidence as those from peer-reviewed articles.

Distribution of TBAc-derived Radioactivity in Rats

Once in the blood of the rat, TBAc is distributed to all major tissues. TBAc does not appear to accumulate extensively in body tissues (Cruzan and Kirkpatrick 2006), and is either exhaled as the parent compound or metabolized.

As part of a series of experiments involving rodent exposure to TBAc, metabolism and pharmacokinetics studies using male Sprague-Dawley rats were reported by Cruzan and Kirkpatrick (2006). Animals (n = 6/group) were exposed by nose-only inhalation to either 100 or 1000 ppm (475 or 4751 mg/m^3, respectively) of carbon-14 radiolabeled TBAc (^{14}C-TBAc) for 6 hours to facilitate the study of TBAc metabolism and elimination. Two animals/group were sacrificed immediately after exposure for analysis of radioactivity in tissues. The remaining 4 animals/group were placed in metabolism cages for collection of feces, urine, and expired air over seven post-exposure days. After the collection period, these animals were sacrificed and analyzed as above.

Once exposure ended, radioactivity was rapidly eliminated, mostly within 24 hours, irrespective of the ^{14}C-TBAc concentration in the air. Low-exposure (100 ppm; 475 mg/m^3) rats excreted most of the calculated TBAc-derived inhaled radioactivity in urine (89% of dose). This calculated dose reflects the amount of radioactivity retained by rats at the end of the exposure period (personal email communication to OEHHA from Dr. Banton, 2017). The remaining TBAc-derived radioactivity was found in feces, expired air, and tissues (2.7, 4.8, and 0.7% of dose, respectively). The primary excretion route of TBAc-derived radioactivity for high-exposure (1000 ppm; 4751 mg/m^3) rats was also urine (69% of dose), but a larger proportion was found in expired air (27% of dose) compared to low-dose animals. The remaining TBAc-derived radioactivity was located in feces and tissues (0.97 and 0.22% of dose, respectively).

Animal tissues from both exposure groups retained very little of the residual radioactivity. In low-exposure rats, mean TBAc-equivalent concentrations of 0.925, 0.328, 0.891, 0.131 and 0.246 µg/g were detected in the nasal tissues, larynx, trachea, lungs, and fat, respectively, while concentrations ranging from 0.084 to 0.170 µg/g were detected in the liver, kidneys, spleen, and blood. In high-exposure rats, TBAc-equivalent concentrations were 1.17 µg/g in fat, and ranged between 0.40 and 0.55 µg/g on average in the liver, kidney and lung tissues. However, concentrations in nasal tissues, larynx, trachea, spleen, and blood were below detection limits.

Lower radioactivity in the tissues of rats from the high versus low exposure group (4.8 and 27%, respectively) was determined by the study authors as an indication of

partial saturation of the absorption and metabolism of TBAc at some concentration below 1000 ppm (4751 mg/m^3).

As noted above, when compared to their low-exposure counterparts, high-exposure rats exhibited lower and higher proportions of available radioactivity in tissues and expired air, respectively. The Cruzan and Kirkpatrick (2006) publication did not specify whether the radioactivity in exhaled air was due to the presence of TBAc or TBA. However, in the original report by Huntingdon Life Sciences Limited (2000b), which was later published by Cruzan and Kirkpatrick (2006), two major radioactive components were detected in expired air from high-dose animals 6 hours post exposure. Huntingdon Life Sciences Limited stated that one component was chromatographically identical to parent TBAc, while the other component was unidentified. According to the authors, co-chromatography of urine with expired air, via high-performance liquid chromatography, showed that the radioactive metabolites detected in exhaled air were not the same compounds detected in urine.

Metabolism and elimination of TBAc in Rats

Rat metabolism of TBAc is thought to take place by both carboxylesterase (CE)- and cytochrome P450 (CYP)-dependent pathways (Cruzan and Kirkpatrick 2006, Bus *et al*., 2015). One major pathway involves hydroxylation of the TBA portion of TBAc to produce 2-hydroxymethyl-isopropyl acetate via oxidation. The second major route involves cleavage of the ester linkage to produce TBA.

Cruzan and Kirkpatrick (2006) have proposed that TBAc metabolism in rats follows two major pathways. The hydroxylation of the TBA portion of TBAc produces 2-hydroxymethyl-isopropyl acetate (U1), which then forms 2-hydroxymethyl-isopropyl acetate glucuronide via glucuronidation (U6) or 2-hydroxyisobutyric acid (HBA; U2), the major urinary metabolite, via oxidation (Figure 3; left side). The second route involves cleavage of the ester linkage to produce TBA (not detected in urine), which formed TBA glucuronide (U4) via glucuronidation or HBA via oxidization (Figure 3; middle). A minor route involved the hydroxylation of the acetate portion of TBAc to produce a *t*-butyl-2-hydroxyacetate intermediate, which formed t-Butyl-2-hydroxyacetate glucuronide (U8) via glucuronidation (Figure 3; right side).

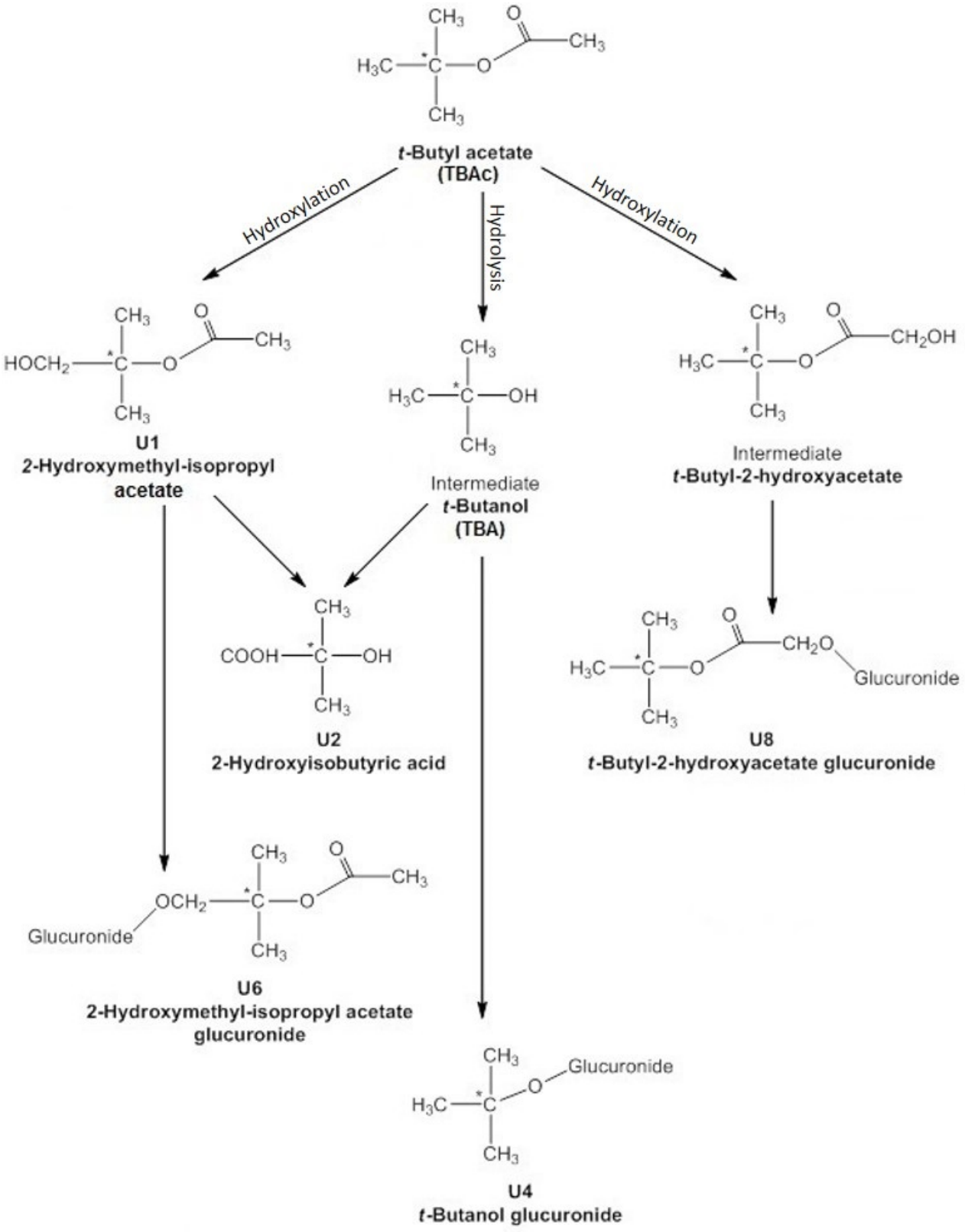

Figure 3. Proposed Metabolic Pathways for *Tertiary*-Butyl Acetate in Rats (adapted from Cruzan and Kirkpatrick, 2006). "*C" indicates a carbon-14 atom.

Four major urinary metabolites (U1, U2, U4, and U6) were detected in the study by Cruzan and Kirkpatrick (2006). During the first 48 hours post exposure, these accounted for 9.3, 45.5, 11.0 and 10.0% of the dose, respectively, in low-dose animals, and 7.9, 38.9, 15.2 and 2.6% of the dose, respectively, in high-dose animals. Three minor urinary metabolites, U3, U7, and U8, accounted for 1 to 1.6% and 0.4 to 1.1 % of the dose in the low-dose and high-dose animals, respectively. U3 and U7 were not identified. Percentages of urinary metabolites detected up to 48 hours after the 6-hour exposures are shown in Table 8.

Table 8. Proportions of radioactivity in urine (0 – 48 hours) after a 6-hour inhalation exposure to ^{14}C-*tertiary*-butyl acetate (TBAc) at 100 or 1000 ppm (adapted from Cruzan and Kirkpatrick, 2006).

Metabolite Pathway (Product)	100 ppm Exposure	1000 ppm Exposure
Hydroxylation of TBAc (U1 + U6)	24.2%	15.8%
Hydrolysis to TBA (U4)	13.8%	22.8%
Either pathway (U2)	57.1%	58.4%
Hydroxylation of acetate (U8)	1.3%	0.6%
Unidentified metabolites (U3 + U7)	3.5%	2.4%

% of total urinary metabolites in 48 hours.
Abbreviations: TBA = *tertiary*-butanol. U1 = 2-hydroxymethyl-isopropyl acetate. U2 = 2-hydroxyisobutyric acid. U4 = TBA glucuronide. U6 = 2-hydroxymethyl-isopropyl acetate glucuronide. U8 = *t*-butyl-2-hydroxyacetate-glucuronide conjugate. These metabolites are illustrated in Figure 3 (above).

Bus *et al.* (2015) have suggested that the hydrolysis of TBAc to TBA in rats is mediated by CEs. Human and mouse data for CE-mediated metabolism of TBAc to TBA are not available. However, there are a variety of CEs in both humans (six isoforms) and mice (20 isoforms) (Jones *et al.*, 2013). These CEs have broad substrate specificity and are distributed within a wide variety of tissues (Redinbo and Potter, 2005), suggesting that this metabolic pathway could be operative in both humans and mice.

TBA and Oxygenated Butyl Ethers (MTBE and ETBE)

MTBE induces tumors in rats: leukemias, lymphomas and Leydig cell testicular tumors after oral exposure, and renal tubular and Leydig cell testicular tumors after inhalation exposure. OEHHA has used these data to develop an IUR for MTBE (OEHHA, 1999b). ETBE induces liver tumors in rats after inhalation exposure (Saito *et al.*, 2013).

CYPs have been shown to metabolize MTBE and ETBE to TBA in rats and humans (Figure 4).

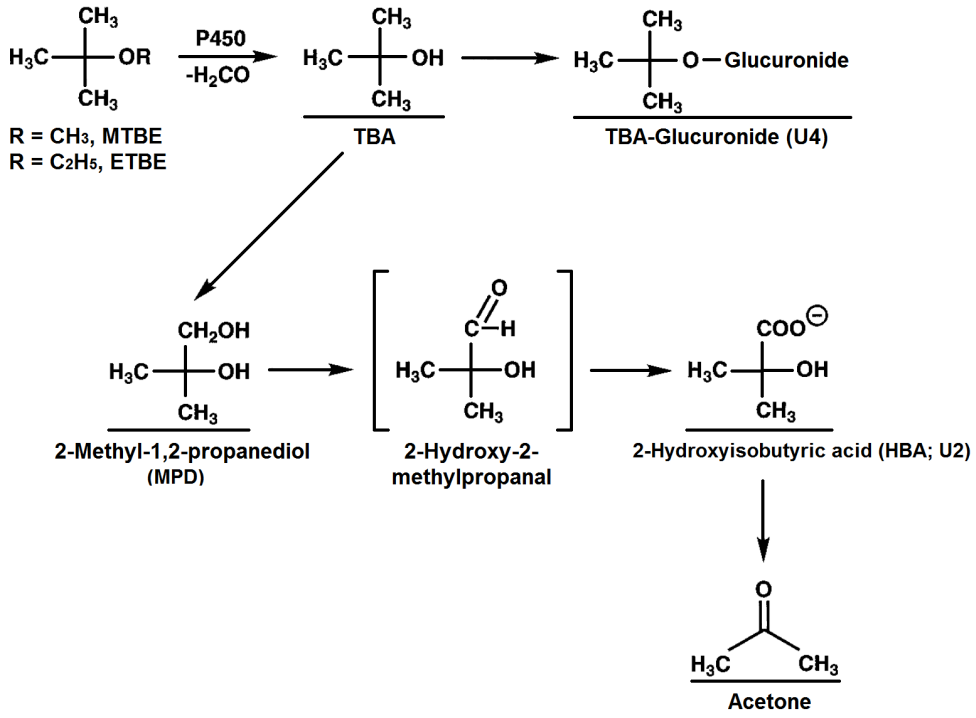

Figure 4. Biotransformation of MTBE and ETBE in rats and humans (adapted from Bernauer *et al*., 1998). Excreted metabolites are underlined.

Those CYPs also metabolize TBAc, making them potential surrogates for comparing TBAc metabolism between rats and humans. MTBE and ETBE PK data are available for both rats and humans. Both compounds are metabolized *in vivo* by CYPs to TBA, aldehydes, and acids in rats and humans. Studies indicate that the metabolism and pharmacokinetics of MTBE, ETBE, and the TBA produced from metabolism of those compounds are reasonably similar in these two species. Less corresponding information was located for mice.

These data also indicate that MTBE-biotransformation and excretion after oral exposure was similar to inhalation exposure suggesting an absence of significant first-pass metabolism of MTBE in the liver.

Distribution of TBA, MTBE and ETBE in Humans

Partition coefficients for TBA were calculated using blood from human volunteers by Nihlén *et al*. (1995). The calculated tissue:blood partition coefficients for TBA were slightly above 1 (from 1.02 to 1.06) for most tissues, except for fat:blood, which was 0.646. The same study evaluated the partition coefficients of oxygenated ethers, including MTBE and ETBA. The study concluded that TBA preferentially distributes in body water, and the ethers distribute uniformly throughout the body with preference for fatty tissues.

Metabolism of MTBE, ETBE and TBA by Rats and Mice after Inhalation Exposures

In a non-peer reviewed study performed by the Chemical Industry Institute of Toxicology (CIIT) (Borghoff and Asgharian, 1996), male and female F344 rats and CD-1 mice (8/sex/concentration) were exposed by nose-only inhalation for 6 hrs to approximately 500, 1750, or 5000 ppm (1805, 6318 and 18,050 mg/m^3) ^{14}C- ETBE vapor. After exposure, eight animals from each species (4 male, 4 female) were sacrificed and the carcasses digested to determine the total radioactivity present. The other 8 animals from each species rats (4 male and 4 female) were placed in individual glass metabolism cages for the collection of excreta for up to 48 hrs following exposure. All samples were analyzed for ^{14}C by liquid scintillation spectrometry.

The majority of ^{14}C-ETBE-derived radioactivity (>90%) was eliminated from all animals by 48 hr at all ^{14}C-ETBE concentrations. Radioactivity from absorbed ^{14}C-ETBE was recovered primarily in the urine and as exhaled volatile organics. Elimination of ^{14}C-ETBE-derived radioactivity in the 500 ppm rats was primarily in urine, while the elimination of ^{14}C-ETBE-derived radioactivity in the 5000 ppm rats was primarily through exhalation of volatile organics. Mice demonstrated a similar relationship, except that in the 5000 ppm group, the elimination of ^{14}C-ETBE-derived radioactivity in urine and exhaled as volatile organics were similar. Both ETBE and TBA were identified in rat and mouse urine samples, with TBA levels being generally greater than ETBE levels in both species. HBA and 2-methyl-1,2-propanediol (MPD) were also identified in both rat and mouse urine samples (exposure groups and relative levels not specified).

Bernauer *et al*. (1998) investigated the biotransformation of MTBE, ETBE, and TBA after inhalation exposure in rats. Male and female Fischer 344 rats (n = 2/sex/exposure) were exposed in whole-body chambers to 2000 ppm 2-carbon-13-labeled-MTBE (2-^{13}C-MTBE; 7210 mg/m^3) or 2-^{13}C-ETBE (8358 mg/m^3) for a single 6-hour period, then placed in individual metabolism cages for 48 hours for collection of urine at 24-hour intervals. Urinary metabolites were detected and identified via ^{13}C nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry.

NMR signal intensities, which are proportional to molar concentrations of the identified metabolites, indicated that HBA > MPD > TBA-sulfate > TBA-glucuronide up to 24 hours post exposure to 2-^{13}C-MTBE. Relative urinary metabolite concentrations from 2-^{13}C-ETBE-exposed rats followed a similar order at the same time-point: MPD > HBA > TBA-sulfate > TBA-glucuronide. Overall, these results suggested to the authors that the major MTBE and ETBE metabolites (HBA and MPD) in rat urine were those that resulted from TBA biotransformation. This conclusion is bolstered by other studies (Amberg *et al*., 1999; Nihlén *et al*., 1999; Amberg *et al*., 2000) in rats and humans that suggest urinary excretion of TBA is low (<5% of the exposed dose) relative to HBA and MPD after MTBE/ETBE inhalation. Urine samples collected at 48 hours post exposure to 2-^{13}C-MTBE contained the same major metabolites as those found at the earlier time-point, but at lower concentrations. It was unclear to OEHHA whether the relative amounts of metabolites remained the same. The only metabolite present in 48-hour urine samples from 2-^{13}C-ETBE-exposed rats was HBA. This latter finding indicated to the authors that HBA was excreted slowly and over a relatively long period after exposure to ETBE.

The information described above indicates that MTBE (in rats) and ETBE (in rats and mice) are metabolized to TBA, which is then further metabolized to compounds such as HBA, which is also observed after TBAc metabolism in rats.

Metabolism of MTBE, ETBE and TBA by Human, Rat, and Mouse Liver Microsomes

Hong *et al*. (1997) conducted metabolic studies on MTBE using human liver microsomes, subcellular fractions that contain membrane-bound drug-metabolizing enzymes including CYPs. Given that CYP2A6 and 2E1 were known to be constitutively expressed in the human liver, and previous studies showed rat microsomal CYPs were involved in metabolism of MTBE, Hong *et al*. (1997) investigated several endpoints. These included the enzymatic activity of human liver microsomes incubated with MTBE and the comparative activity of human, rat, and mouse microsomes and cytosol incubated with MTBE.

Human microsomes and cytosol were isolated from non-cancerous and morphologically normal liver tissue samples obtained from liver cancer patients (n = 8; age 37 – 80 years; sex not stated) in the United States. Microsomal and cytosolic fractions of liver tissues collected from male Sprague-Dawley rats (n = 5; 10 weeks old) and female A/J mice (n = 4; 7 weeks old) were also prepared.

Study data indicated that human liver microsomes were capable of metabolizing MTBE to TBA when incubated with 1 mM MTBE and a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-generating system at 37 °C for 30 minutes. (These parameters were repeated for all experiments.) The tested human microsomes were active (*i.e.* they converted MTBE to TBA) at rates ranging from 86 - 176 pmol MTBE/minute/mg protein. Additional tests of microsomal samples incubated without the CYP-required NADPH-generating system, or in the presence of carbon

monoxide, a CYP inhibitor, showed non-detectable and 80.5% reduced activities, respectively, supporting the role of CYPs in human MTBE metabolism.

Hong *et al.* (1997) also reported that human microsomal MTBE metabolism to TBA was shown to be approximately half that of the rat and mouse. Mean rates ± standard deviation were 124.9 ± 11.3, 284 ± 14, and 288 ± 29 pmol/min/mg protein, respectively. The study results suggested that both human and rodent liver CYPs play important roles in the metabolism of MTBE for their respective species.

Human Metabolism of MTBE, ETBE, and TBA after Inhalation Exposure

Human data on the *in vivo* metabolism and elimination of MTBE, ETBE and TBA was obtained by Nihlén *et al*. (1998; 1999). Human volunteers (n = 8 or 10) were exposed to 5, 25, or 50 ppm MTBE (18.0, 90.1, 180 mg/m^3) or ETBE (20.9, 104.5, and 209 mg/m^3) by inhalation during 2 hours of light exercise. TBA was detected in blood samples at levels similar to that of MTBE and ETBE. The urinary excretion of TBA was less than 1% of the absorption of MTBE or ETBE, indicating TBA conversion to other metabolites.

Amberg *et al.* (1999) exposed human volunteers (3 males and 3 females) and rats (5 each, males and females) to 4 and 40 ppm (14.4 and 144 mg/m^3) MTBE for 4 hrs. Urine samples from rats and humans were collected for 72 hrs in 6-hr intervals, and blood samples were taken at regular intervals for 48 h. MTBE and the MTBE metabolites TBA, HBA, and MPD were quantified in urine, and MTBE and TBA were quantified in blood samples.

After the end of the exposure period, the MTBE blood concentrations in the 4 ppm exposure groups were 2.3 µM in rats and 1.9 µM in humans, and 5.9 µM in rats and 6.7 µM in humans in the 40 ppm (144 mg/m^3) MTBE exposure groups. The TBA blood concentrations were 2.9 µM in rats and 2.6 µM in humans in the 4 ppm (14.4 mg/m^3) exposure groups and 36.7 µM in rats and 21.8 µM in humans in the 40 ppm (144 mg/m^3) exposure groups.

HBA was recovered in both rats and humans as a major urinary metabolite; TBA and MPD were minor urinary metabolites. All urinary metabolites of MTBE were rapidly eliminated in both species after the end of the MTBE exposure.

Amberg *et al*. (2000) exposed six human volunteers (n = 3/sex; 28 ± 2 years old) to 18.8 or 170 mg/m^3 (4.5 or 40.7 ppm) ETBE for a total of eight hours over two exposure periods. Exposures were separated by four weeks of recovery; each exposure lasted 4 hours; and volunteers were exposed to the same concentration during each exposure. Urine was collected at 6-hour intervals for 72 hours following each exposure, and total metabolites (conjugated and unconjugated) were measured.

TBA and two of its metabolites, HBA and MPD, were identified in urine. The predominant urinary metabolite was HBA, excreted at 5–10 times the amount of MPD, and 12–18 times the amount of TBA.

Human and Rat Metabolism of MTBE/ETBE and Elimination of TBA after Oral Exposure

In one human individual given carbon-13 labeled TBA (^{13}C-TBA) orally at a dose of 5 mg/kg body weight (BW), major urinary metabolites detected by ^{13}C-NMR spectroscopy included HBA and MPD (Bernauer *et al.*, 1998). Unconjugated TBA and TBA-glucuronide were present as minor metabolites. Traces of the presumed TBA-sulfate were also present. Additional experiments in rats exposed to 2000 ppm 2-^{13}C-MTBE (7210 mg/m^3) or 2-^{13}C-ETBE (8358 mg/m^3) for a single 6-hour period via inhalation, or ^{13}C-TBA in corn oil via gavage (250 mg/kg BW) also identified HBA and MPD as major urinary metabolites (Bernauer *et al.*, 1998). HBA has also been identified as a major urinary metabolite of TBAc (Cruzan and Kirkpatrick, 2006).

Because the same urinary metabolites were detected in the TBA-exposed human volunteer, TBA-exposed rats, MTBE-exposed rats, and ETBE-exposed rats, Bernauer *et al.* (1998) suggested that the biotransformation of MTBE and ETBE may be qualitatively identical in rats and humans. Based upon their findings, they also concluded that MTBE and ETBE are extensively metabolized by further oxidation reactions. Indeed, oxidation of MTBE/ETBE yields TBA which is oxidized in subsequent reactions to form the major urinary metabolites, HBA and MPD (Figure 4). This was supported by the research by Hong *et al.* (1997), which showed that the human liver was able to oxidize MTBE and ETBE to TBA.

Amberg *et al.* (2001) exposed human volunteers (3 males and 3 females, identical individuals, exposures performed 4 weeks apart) to 5 and 15 mg ^{13}C-MTBE dissolved in water. Urine and blood samples from the volunteers were collected for 96 h post-treatment in 6-hr intervals and in intervals for 24 hr, respectively. MTBE, TBA, HBA and MPD were quantified in urine, and MTBE and TBA were quantified in blood. MTBE and TBA were also quantified in exhaled air in a study of three male volunteers given 15 mg MTBE in water. MTBE blood concentrations were 0.10 and 0.69 µM after 5 and 15 mg MTBE, respectively. TBA blood concentrations were 0.45 and 1.82 µM after 5 and 15 mg MTBE, respectively.

Approximately 30% of the administered MTBE was cleared by exhalation as unchanged MTBE and as TBA. MTBE exhalation was rapid, maximal MTBE concentrations (100 nmol/l) in exhaled air were achieved within 10–20 minutes, and clearance of MTBE by exhalation paralleled clearance of MTBE from blood.

HBA was the major urinary metabolite, and TBA and MPD were minor urinary metabolites. Approximately 50% of the administered MTBE was recovered in urine, and another 30% was recovered in exhaled air as unchanged MTBE and TBA. The authors stated that “the obtained data indicate that MTBE-biotransformation and excretion after oral exposure is similar to inhalation exposure and suggest the

absence of significant first-pass metabolism of MTBE in the liver after oral administration" and "possible adverse effects of MTBE in humans are thus expected to be independent of route of exposure."

In summary, MTBE is metabolized to TBA, HBA and MPD in both rats and humans after oral exposure. These metabolites are the same as those seen after MTBE inhalation exposure. TBA also appears in the exhaled breath of humans after both oral intake and inhalation exposure, suggesting that MTBE lacks a significant first pass effect in humans. Direct data on the status of TBA first pass metabolism in humans is not available, but the MTBE data suggests that TBA may also not experience significant first pass metabolism in humans. The MTBE data also indicates that MTBE toxicity in humans is probably independent of the route of exposure, which suggests that TBAc toxicity in humans may also be route-independent.

Cancer Bioassays

Tertiary-Butanol

The National Toxicology Program (NTP) conducted a two-year cancer bioassay using Fischer 344 (F344) rats and $B6C3F_1$ mice exposed to TBA in drinking water (NTP, 1995; Cirvello *et al*., 1995).

Groups of 60 F344 rats were administered daily doses of TBA via drinking water of 0, 1.25, 2.5 or 5 mg/mL (approximately 0, 90, 200, and 420 mg/kg-day) in males and 0, 2.5, 5 or 10 mg/mL (approximately 0, 180, 330, and 650 mg/kg-day) in females. Ten animals in each group were sacrificed at 15 months for evaluation; the remaining animals were exposed until the study was terminated at 103 weeks. The high dose groups of both sexes experienced decreased survival. The two-year survival incidence for males was 10/50, 6/50, 4/50, and 1/50, and the mean survival was 618, 625, 598 and 596 days, respectively, for the control, low-, mid- and high-dose groups (Table 9).

Table 9. Survival of rats in the 2-Year drinking water study of *tertiary*-butanol (NTP 1995).

Males	**0 mg/mL**	**1.25 mg/mL**	**2.5 mg/mL**	**5 mg/mL**
Initial number of animals	60	60	60	60
15-month interim evaluation[a]	10	10	10	10
Moribund	31	35	41	46
Natural Deaths	9	9	5	3
Animals surviving to study termination	10	6	4	1
Probability of survival at end of study[b] (%)	20	12	8	2
Mean survival (days)[c]	618	625	598	596
Survival analysis[d]	$p = 0.001$	$p = 0.853$	$p = 0.091$	$p = 0.010$
Females	**0 mg/mL**	**2.5 mg/mL**	**5 mg/mL**	**10 mg/mL**
Initial number of animals	60	60	60	60
15-month interim evaluation[a]	10	10	10	10
Moribund	15	18	21	35
Natural Deaths	7	8	7	3
Animals surviving to study termination	28	24	22	12
Probability of survival at end of study (%)	56	48	44	24
Mean survival (days)	669	649	642	643
Survival analysis	$p = 0.004$	$p = 0.475$	$p = 0.334$	$p = 0.006$

[a]Interim evaluation censored from survival analyses.
[b]Probabilities of survival are Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice.
[c]Mean survival represents the mean of all deaths (uncensored, censored, and terminal sacrifice).
[d]For survival analyses, the results of the life table trend tests (Tarone, 1975) are in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.

A dose-related decrease in body weight gain was observed. All treated groups of females showed a dose-related increase in kidney weight at the 15-month evaluation. Males exhibited increased kidney weight at the mid- and high-doses. Nephropathy was seen in all groups of treated females and caused early mortality in high exposure groups.

After performing the initial rat histopathological evaluation, NTP decided to evaluate step sections from the residual kidney wet tissue from the male rats in the 2-year studies (1995). Taking sections at specified depths from a tissue block (*e.g.*, every 50 microns) creates a step section. The step-sectioning procedure is a more sensitive procedure for detecting tumors in the tissue under examination. NTP staff

discussed the differences between standard pathology sectioning and step-sectioning in the 1995 report, and suggested that the two procedures were analogous to a partial evaluation and a definitive evaluation, respectively.

This action was taken because microscopic examination of the original male rat kidney sections showed an equivocal increase in renal tubule adenomas or carcinomas and increased renal tubule proliferative lesions. NTP believed that performing a step section evaluation would provide a more sensitive tumor incidence evaluation. The sections were taken in 1 mm steps throughout the kidney wet tissue remaining after the standard sections had been taken. NTP made an average of eight additional step sections per kidney.

By the 24-month termination of the study, combined adenomas and carcinomas of the renal tubules had been found in 8/50, 13/50, 19/50, and 13/50 of the control, low-, mid- and high-dose males, respectively (step section evaluation) (Table 10). This tumor incidence included animals that died before study termination. The increased incidence in the mid-dose group was statistically significant (p = 0.012) by Fisher's exact test.

Table 10. Increased tumor incidences in Fischer 344 male rats and male and female B6C3F$_1$ mice exposed to *tertiary*-butanol in drinking water (NTP, 1995).

Sex, species	Tumor type	Administered dose (mg/mL)	Calculated dose (mg/kg-day)	Tumor incidence
Male rats	Renal tubule adenomas/carcinomas (single section	0	0	1/50
		1.25	90	3/50
		2.5	200	4/50
		5	420	3/50
Male rats	Renal tubule adenomas/carcinomas (step sectioned)	0	0	8/50
		1.25	90	13/50
		2.5	200	19/50**
		5	420	13/50
Male mice	Thyroid follicular cell adenomas or carcinomas	0	0	1/60
		5	540	0/59
		10	1040	4/59
		20	2070	2/57
Female mice	Thyroid follicular cell adenomas	0	0	2/58$^+$
		5	510	3/60
		10	1020	2/59
		20	2110	9/59*

Fisher exact test pairwise comparison with controls: * p = 0.028; ** p = 0.012
Cochran-Armitage trend test for dose response: $^+$ p = 0.007

The pathogenesis of proliferative lesions of renal tubule epithelium is thought to proceed from hyperplasia to adenoma to carcinoma (Cirvello *et al*., 1995). Renal tubule hyperplasia was significantly increased ($p \leq 0.01$) in the male rat high dose treatment group (as determined by either step section evaluation or standard and step section evaluations combined). Control male rat renal tubule adenoma incidence was 2/327, and carcinoma incidence was 0/327 in the six studies comprising the 1984 – 1994 NTP male F344 rat historical control database for drinking water studies, indicating the rarity of these neoplasms in male F344 rats (NTP, 2017a). The renal tubule adenoma incidence in the concurrent control group of male rats in the TBA NTP (1995) study was considerably greater than the corresponding historical control incidence. Those historical controls were evaluated using a single section histopathological evaluation. An evaluation of those same controls using step sectioning could have identified a higher renal tubule tumor incidence due to greater tumor detection sensitivity. However, NTP (1995) cited Eustis *et al.* (1994), who found that in previous NTP studies using this method of extended evaluation, the incidence of carcinoma in male rats was 1/599 among 12 control groups.

Groups of 60 $B6C3F_1$ mice of each sex were administered TBA in drinking water at doses of 0, 5, 10 or 20 mg/mL (approximately 0, 540, 1040, and 2070 mg/kg-day in males and 0, 510, 1020, and 2110 mg/kg-day in females). Reduced survival was observed in the high dose groups (Table 11, below).

Table 11. Survival of mice in the 2-year drinking water study of *tertiary*-butanol (NTP 1995).

Males	**0 mg/mL**	**5 mg/mL**	**10 mg/mL**	**20 mg/mL**
Initial number of animals	60	60	60	60
Accidental deaths[a]	0	2	1	0
Moribund	20	14	19	21
Natural deaths	13	8	6	22
Animals surviving to study termination	27	36	34	17
Probability of survival at end of study[b] (%)	45	62	58	28
Mean survival (days)[c]	676	665	678	538
Survival analysis[d]	$p < 0.001$	$p = 0.077$N	$p = 0.228$N	$p = 0.007$
Females	**0 mg/mL**	**5 mg/mL**	**10 mg/mL**	**20 mg/mL**
Initial number of animals	60	60	60	60
Accidental deaths[a]	1	3	2	2
Moribund	13	14	13	12
Natural deaths	10	8	4	4
Animals surviving to study termination	36	35	41[e]	42
Probability of survival at end of study (%)	61	61	71	72
Mean survival (days)	657	677	682	682
Survival analysis	$p = 0.121$N	$p = 0.924$N	$p = 0.298$N	$p = 0.198$N

[a]Censored from survival analyses.
[b]Probabilities of survival are Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice.
[c]Mean survival represents the mean of all deaths (uncensored, censored, and terminal sacrifice).
[d]For survival analyses, the results of the life table trend tests (Tarone, 1975) are in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.
[e]Includes one animal that died during the last week of the study.

In the 1995 NTP mouse bioassay, incidence of thyroid follicular cell hyperplasia was significantly elevated in all treatment groups of males (5/60, 18/59, 15/59, 18/57) and in the mid- and high-dose groups of females (19/58, 28/60, 33/59, 47/59). A significant dose-dependent increase in follicular cell adenomas was observed in female mice ($p = 0.007$, Cochran-Armitage trend test), with adenoma incidence significantly increased compared to controls by pairwise comparison in high dose females (9/59, $p = 0.028$, Fisher exact test; Table 10). NTP (1995) stated that "proliferation of thyroid gland follicular cells is generally considered to follow a progression from hyperplasia to adenoma and carcinoma".

Thyroid follicular cell adenoma incidence was also increased in the male mouse 10 mg/mL exposure group. This increased tumor incidence was not statistically significant compared to the concurrent control group, but NTP (1995) noted that the 7% tumor incidence in this group exceeded the highest rate of 2% observed in NTP historical controls for drinking water studies. NTP (1995) also noted that reduced survival in the 20 mg/mL exposure group may have limited the ability to detect a carcinogenic effect. For these reasons, NTP considered the increased thyroid follicular cell adenoma incidence in male mice to be equivocal evidence of carcinogenic activity. Chronic urinary bladder inflammation was seen in both sexes at the high dose, but no urinary bladder neoplasms were observed.

NTP (1995) concluded the increased incidence of renal tubule adenoma or carcinoma, combined, in male rats and of thyroid gland follicular cell adenoma in female mice is evidence of a carcinogenic response to TBA.

IV. SUPPORTING DATA

TBAc is metabolized in rats to TBA (Groth and Freundt, 1994; Huntingdon Life Sciences Ltd., 2000b). Oral TBA exposure has been demonstrated to induce renal tumors in male rats and thyroid follicular cell tumors in female mice (NTP, 1995).

Male rat kidney tumors observed after oral TBA exposure have been proposed to be related to a TBA-α2u-globulin interaction resulting in α2u-globulin nephropathy (Borghoff *et al.*, 2001; McGregor and Hard, 2001; Hard *et al.*, 2011). The relevance of the renal tumors in rats induced by this potential mode of action to human cancer risk has been questioned. The defining feature of α2u nephropathy is the rapid accumulation of protein (or hyaline) droplets in the renal proximal tubule cells of many strains of male rats following chemical treatment. Female rats do not appear to produce α2u. α2u is usually degraded in the lysosomal compartment of the renal proximal tubule cells. It is believed that binding of α2u to chemicals results in an impairment of the lysosomal degradation of this protein, leading to an accumulation of protein droplets containing α2u. Protein droplet accumulation occurs rapidly, and leads to progressive renal injury, characterized by single cell degeneration and necrosis in the renal proximal tubule. Renal proximal tubule necrosis causes compensatory cell proliferation in the renal cortex. Cessation of cell proliferation occurs shortly after stopping acute exposures to α2u-inducers, and a restoration of normal renal architecture can occur. Chronic exposure to α2u-inducers leads to linear papillary mineralization. Atypical hyperplastic cellular foci may eventually be observed in the proximal tubules, and these foci may eventually progress to renal adenomas and carcinomas (Swenberg and Lehman-McKeeman, 1999).

We provide information in the following discussion relevant to this possible mode of action of TBA-induced kidney tumor formation. Further, we provide some information on whether the thyroid tumors may be the result of hyperplasia due to thyroid hormone disruption. A discussion of the data supporting any particular mode of action is provided in Section V, Cancer Hazard Identification, below.

Biochemical Effects and Cell Proliferation

Tertiary-Butyl Acetate

Male and female CD(SD) rats and CD1(IGR) mice (10 animals/sex/exposure group for rats, 40 animals/sex/exposure group for mice) were exposed to TBAc by inhalation at concentrations of 0, 100, 400 or 1600 ppm for 6 hours/day, 7 days/week for 13 weeks by Faber *et al.* (2014). These concentrations were described as being approximately equal to 0, 135, 540 and 2160 mg/kg-day for rats, and 0, 237, 948 and 3792 mg/kg-day for mice.

Absolute rat kidney weights were significantly increased ($p < 0.05$) in the male 1600 ppm exposure group compared to controls. Relative rat kidney weights (relative to body weight) were significantly increased ($p < 0.05$) in all male exposure groups and in the 1600 ppm female exposure group compared to controls. The authors stated that renal α2u accumulation was consistent throughout the male rat TBAc exposure groups as measured by both immunohistopathology and enzyme-linked immunosorbent assay (ELISA) analysis, while tubular basophilia demonstrated a slightly higher incidence and severity in the 1600 ppm exposure group. However, data showing the statistical significance were not provided for any of these endpoints. Additionally, a graphical depiction of male rat kidney α2u levels (Figure 5) suggests that there was likely no significant difference between the TBAc exposure groups, and possibly no significant difference between some of the TBAc exposure groups and the control group. The authors stated that TBAc exposure did not cause increases in rat kidney tubular cell proliferation as measured by proliferating cell nuclear antigen (PCNA) assessment.

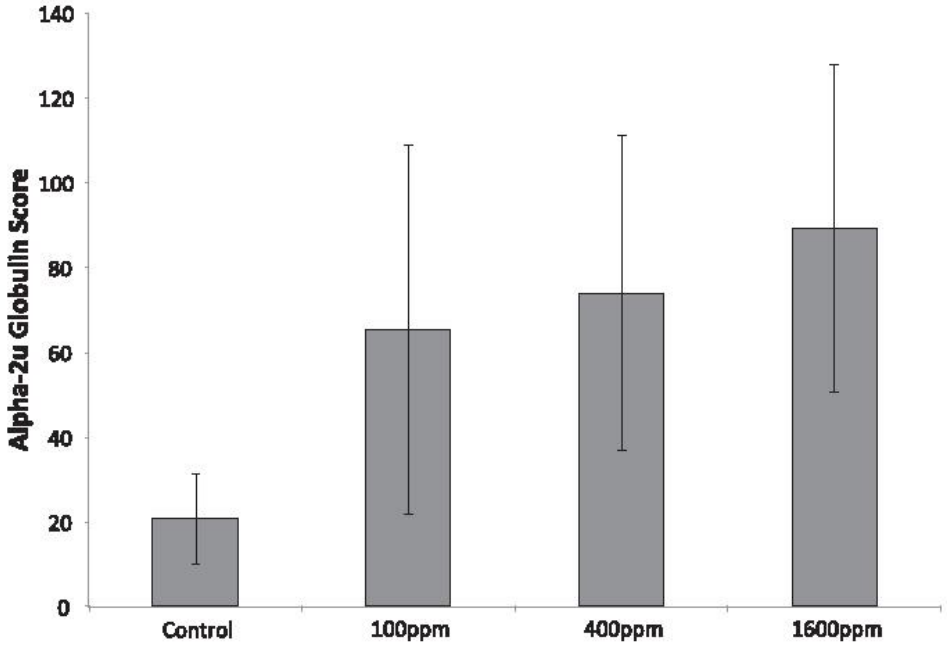

Figure 5. The effect of TBAc exposure on α-2u-globulin score (ng/mg of total protein) in male rats during a 13-week inhalation study. (Figure 2 from Faber *et al.*, 2014), reproduced unchanged by permission)

The authors provided absolute and relative liver weights, absolute thyroid/parathyroid gland weights and thyroid-stimulating hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4) blood levels in mice. The data table legend (Table 6 in Faber *et al.*, 2014) indicated that the mice had been exposed to TBAc for 20 weeks. However, the test description for these data indicated that the TSH, T_3 and T_4 data were collected at study week 4, and the thyroid/parathyroid gland weights were obtained at study week 13. No significant changes in TSH or T_3 levels were observed in TBAc-exposed male or female mice compared to controls. Significantly decreased T_4 levels ($p < 0.05$) were only noted in the male 1600 ppm mouse exposure group. The authors did not observe significant thyroid gland histopathological changes or alterations in thyroid/parathyroid gland weights in the TBAc-exposed mice compared to controls. Absolute liver weights were significantly increased in the 1600 ppm female mouse exposure group (23% increase compared to controls; $p < 0.05$). Relative liver weights were significantly increased in the male and female 1600 ppm exposure groups (11.6% and 20.4% increase compared to controls, respectively; $p < 0.05$).

Tertiary-Butanol

The NTP (1995) conducted a 13-week TBA drinking water exposure study in male and female F344 rats and $B6C3F_1$ mice (10 animals/sex/species). TBA drinking water exposure levels were 0, 2.5, 5, 10, 20 or 40 mg/mL (0, 0.25, 0.5, 1, 2 and 4%).

Calculated doses were 0, 230, 490, 840, 1520 and 3610 mg/kg-day for male rats and 0, 290, 590, 850, 1560 and 3620 mg/kg-day for female rats. Calculated doses for male mice were 0, 350, 640, 1590, 3940 and 8210 mg/kg-day and 0, 500, 820, 1660, 6430 and 11,620 mg/kg-day for female mice.

All male rats in the 40 mg/mL exposure group died prior to study termination. Both absolute and relative (to BW) kidney weights were significantly increased at all exposure levels for both male and female rats.

No increases in absolute liver weights were noted in either male or female TBA-exposed mice. Increases in relative liver weights were noted in the 20 and 40 mg/mL exposure group in male mice, and in the 40 mg/mL exposure group in female mice.

Lindamood *et al.* (1992) presented selected data from the NTP (1995) 13-week drinking water study of TBA in male and female F344 rats. Comparisons between the vehicle control group and all treated groups were performed by the authors using a Kruskal-Wallis rank-based nonparametric test. Pairwise comparisons between the vehicle control group and treatment groups were evaluated using the Mann-Whitney U-test. Statistically significant ($p < 0.05$) increases were noted for hyaline droplet deposition (5 - 20 mg/mL treatment groups), hyaline crystal deposition and nephropathy (2.5 - 20 mg/mL treatment groups) (Table 12).

Numerical grades for hyaline droplets were defined as follows: 0 - no droplets observed; 1 - droplets occupy less than 30% of the proximal tubule cytoplasm; 2 - droplets occupy 31-60% of the proximal tubule cytoplasm; 3 - droplets occupy greater than 60% of the proximal tubule cytoplasm.

Hyaline crystals included crystalline and rhomboid shapes and were graded as: 0 - not found; 1 - rare; 2 – frequent; 3 - very frequent.

Nephropathy was graded as: 0 - no nephropathy observed; 1 - occasional nephrons with tubular regeneration, tubular atrophy, and/or slightly thickened basement membranes; 2 - several nephrons showing changes in grade 1; 3 - numerous nephrons with changes in grade I along with distorted and dilated tubules and occasional protein casts.

PCNA staining, a measure of cell proliferation, was assessed by counting the number of S-phase labeled nuclei from proximal tubule epithelial cells in 20 microscopic fields representing a total of 0.154 mm^2 of renal cortex. The tissue area examined contained approximately 2000 epithelial cells from the PI, P2, and P3 segments of the proximal tubule.

Table 12. Results of special microscopic examination of kidney sections from male rats in the 1995 NTP subchronic studies of *tertiary*-butanol (Lindamood *et al.* 1992).

Doses (%)	0	0.25	0.5	1.0	2.0	4.0
Hyaline Droplet	1 (1-2)* $p = 0.0001^a$	1.5 (1-2) $p = 0.3736$	2 (1-3) $p = 0.0067$	2 (2-3) $p = 0.0011$	2 (1-3) $p = 0.0056$	0 (0-0) $p = 0.0001$
Hyaline Crystals	0.5 (0-1)* $p = 0.0001^a$	2 (1-2) $p = 0.0001$	3 (2-3) $p = 0.0001$	3 (2-3) $p = 0.0001$	3 (2-3) $p = 0.0001$	0 (0-0) $p = 0.0118$
Nephropathy	1 (0-1)* $p = 0.001^a$	2 (1-2) $p = 0.0024$	3 (2-3) $p = 0.0001$	3 (2-3) $p = 0.0001$	3 (2-3) $p = 0.0001$	1 (0-2) $p = 0.7518$
PCNA	4 (1-9)* $p = 0.0016^a$	5.5 (1-11) $p = 0.4436$	3 (0-9) $p = 0.1543$	7.5 (3-15) $p = 0.0511$	9.5 (3-11) $p = 0.0215$	0 (0-14) $p = 0.0370$

*Values are the median severity grade for the indicated parameter for all rats in the dose group, followed in parentheses by the range of values among individual rats in that group.
[a]Probabilities associated with the vehicle control group were calculated by the authors using the Kruskal-Wallis test. For all other probabilities, *p*-values were calculated by the authors from comparison of the respective group versus the vehicle control using a Mann-Whitney U test with the Z-scores corrected for ties.
Abbreviation: PCNA – proliferating cell nuclear antigen

NTP (1995) reported similar increases in hyaline droplet deposition, but did not report statistical analysis data. Increases in the median number of renal proximal tubular cell nuclei in S-phase (Deoxyribonucleic Acid (DNA) replication phase) as measured by PCNA incorporation were reported in the 1 and 2% exposure groups by Lindamood *et al.* (1992), with the increase in the 2% group being statistically significant ($p < 0.05$; Table 12). NTP (1995) did not report these data.

Takahashi *et al.* (1993) also reported an analysis of male rat renal cortical sections from the NTP (1995) 13-week TBA drinking water exposure study. These data appear to be the same as reported in Lindamood *et al.* (1992), but presented in graphical form rather than numeric form.

Borghoff *et al.* (2001) studied the potential induction of α2u nephropathy and enhanced renal cell proliferation by TBA and TBA renal dosimetry in male and female F344 rats. Male and female F344 rats were exposed to 0, 250, 450, or 1750 ppm TBA vapors 6 hours/day for 10 consecutive days to assess α2u nephropathy (5 animals/sex/concentration) and renal cell proliferation (5 animals/sex/concentration). TBA dosimetry was assessed using male and female F344 rats following single or repeated (8-day) exposures to target concentrations of 250, 450, or 1750 ppm TBA (3 animals/sex/exposure concentration/time point) for 6 hours/day. Rats were sacrificed at various time points following exposure (2, 4, 6, 8, and 16 hours) to measure levels of TBA in the liver, kidney, and blood and at 1 and 8 days to evaluate the dosimetry of TBA following either single or repeated exposures. Animals evaluated for cell proliferation were subcutaneously implanted with an osmotic pump

for delivery of 5-bromo-2'-deoxyuridine (BrdU). Incorporation of BrdU into renal cell DNA was used as a measure of cell proliferation.

Relative kidney weights (percentage of body weight) were increased in male rats exposed to 1750 ppm and in female rats exposed to 450 and 1750 ppm TBA compared to controls ($p < 0.05$). A statistically significant, concentration-dependent positive trend ($p < 0.05$) for the accumulation of protein droplets in the renal proximal tubule was noted in male but not female rats exposed to TBA. This increase was statistically significant in the male 1750 ppm exposure group ($p < 0.01$) compared to controls. Immunohistochemical staining of protein droplets for α2u in control and TBA-exposed male rat kidney sections within renal proximal tubules indicated α2u incorporation. Staining for α2u was slightly greater in male rats exposed to TBA as compared to control male rats. However, the authors stated that no TBA exposure-related increase in α2u staining intensity in male rats was noted. Kidneys from control or TBA-exposed female rats did not stain positive for α2u. Renal cytosol α2u concentrations in male rats were measured using an ELISA assay. A significant increase in α2u concentration was noted in the 1750 ppm group ($p < 0.05$) compared to controls, in contrast to the α2u staining evaluation, where no TBA exposure-related increased staining was noted. However, very little increase in α2u concentration if any was noted in the 250 and 450 ppm groups. The renal α2u concentration in the 1750 ppm group compared to controls also appears to be substantially less than that observed for the α2u-inducer, 2,2,4-trimethylpentane (TMP; Prescott-Matthews *et al.*, 1997).

A statistically significant ($p < 0.05$) increase in cell proliferation expressed as a labeling index (percent of cells in S-phase) in the renal proximal tubule epithelial cells was observed in all groups of TBA-exposed male rats as compared to control males (Figure 6). No similar significant differences in cell proliferation between TBA-exposed and control female rats were noted. The authors stated that renal α2u concentration was positively correlated with cell proliferation in male rat kidney (linear regression analysis, $r^2 = 0.70$). The regression analysis appears to have been done using group means and standard deviations, but the authors were not specific on this point. The authors also stated that “the concentration of α2u in the kidneys of male rats exposed to 250 or 450 ppm TBA was not significantly increased compared to control rats” (Figure 7).

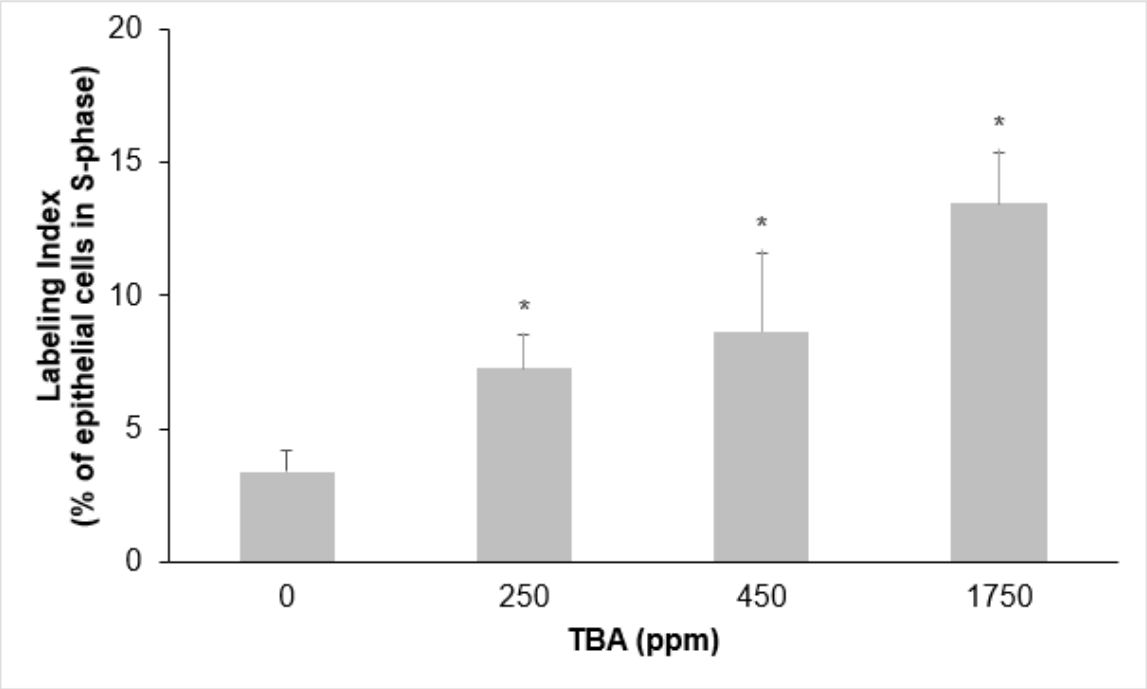

Figure 6. Cell proliferation as measured by quantification of the labeling index in male rats exposed to TBA for 10 consecutive days. Labeling index is a measure of the mitotic activity of a cell population, defined as the number of cells in the S-phase of the growth cycle divided by the total cells in the population*Statistically significant compared to controls (Tukey-Kramer one-way ANOVA; $p < 0.05$; adapted from Figure 4, Borghoff *et al.*, 2001).

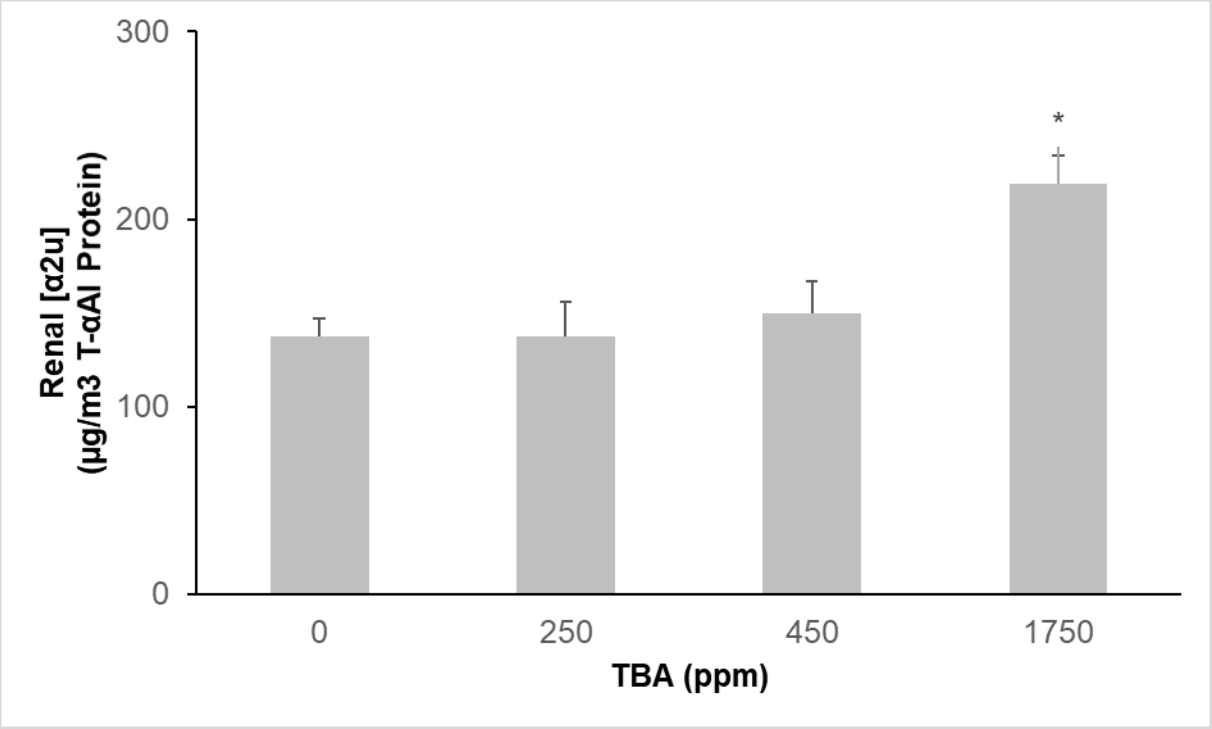

Figure 7. Renal α2u concentration from male rats exposed to TBA as measured by Enzyme-Linked Immunosorbent Assay (ELISA). *Statistically significant compared to control male rats (Tukey-Kramer one-way ANOVA; $p < 0.05$; adapted from Figure 5, Borghoff *et al.*, 2001).

The authors noted these TBA concentrations significantly ($p < 0.05$) increased male rat renal tubule cell proliferation (Figure 6). OEHHA believes the above data indicate that TBA induces male rat renal tubule cell proliferation at concentrations that do not increase renal α2u concentrations, and that increased renal α2u concentrations are not causing renal tubule cell proliferation. Increased renal cell proliferation appears to be solely dependent on TBA concentration. This contrasts with the authors' conclusion that "these data suggest that TBA causes α2u-N [α2-u nephropathy] in male rats, which is responsible for the male rat-specific increase in renal cell proliferation."

The TBA kidney-blood concentration ratios at 2, 4, and 6 hours post exposure were similar (close to a 1:1 ratio) in female rats following either 1 or 8 days exposure. TBA kidney-blood concentration ratios were not provided for female rats at 16 hours post exposure for all concentrations and exposure durations, or for male rats exposed to 250 and 450 ppm but not 1750 ppm TBA for both exposure durations. TBA liver-blood ratios tended to be higher post exposure in male rats exposed for 8 days compared to those exposed for 1 day. Male rat TBA kidney-blood ratios tended to be higher at all concentrations and sampling points after 1 and 8 days of exposure than those of female rats. The exception to this appeared to be the 1 day 1750 ppm exposure groups, where male and female TBA kidney-blood ratios appeared to be similar at 2, 4 and 6 but not 16 hours post exposure. Female rat TBA kidney: blood

ratios tended to remain the same or decrease from 2 to 6 hours post exposure after either 1 or 8 days of exposure. In contrast, male rat TBA kidney-blood ratios tended to increase from 2 to 6 hours post exposure after either 1 or 8 days of exposure. TBA liver-blood ratios were approximately the same for both male and female rats at all post-exposure sampling time points after 8 days of TBA exposure; liver-blood ratio values were not provided for the 16 hours post-exposure time point.

Williams and Borghoff (2001) investigated the binding characteristics of TBA to α2u in male and female F344 rats. Animals used for α2u binding determinations (4/sex/group) were dosed once with 500 mg/kg TBA, 500 mg/kg ^{14}C-TBA, or vehicle (corn oil) by gavage. Animals used for TBA dosimetry determinations (3/sex/group) were dosed once with 500 mg/kg ^{14}C-TBA, or corn oil (vehicle) by gavage.

Kidney TBA levels were increased in male animals compared to female animals ($p \leq 0.06$), and liver, blood and urine TBA levels were significantly increased in male animals compared to female animals. However, the kidney-blood ratios were not substantially different between male and female animals (1.86 and 2.03, respectively).

Renal cytosolic α2u concentrations were significantly increased ($p = 0.0003$) in TBA-treated male rats compared to controls (311.3 mg and 180.4 mg α2u/mg total protein, respectively). Gel filtration analysis of kidney cytosol indicated that an α2u protein standard co-eluted with the low-molecular-weight protein fraction (LMWPF). Anion-exchange chromatography demonstrated that ^{14}C-TBA-derived radioactivity co-eluted with α2u from male kidney cytosol. Incubation of *d*-limonene oxide (a known high affinity α2u ligand) with LMWPF isolated from ^{14}C-TBA-treated male rat kidneys displaced ^{14}C-TBA-derived radioactivity. Gas chromatography-mass spectrometry analysis confirmed that TBA was present in this LMWPF. Dialysis with sodium dodecyl sulfate (SDS) caused loss of ^{14}C-TBA-derived radioactivity from the LMWPF fraction; this did not occur after dialysis without SDS, suggesting that TBA binding to α2u is reversible.

Blanck *et al.* (2010) exposed groups of 15 female B6C3F$_1$ mice to concentrations of 0, 2 or 20 mg/mL TBA in drinking water for either 3 or 14 days. The approximate exposure levels for the 2 and 20 mg/mL exposure groups were 344 and 818 mg/kg-day, respectively, for animals exposed for 3 days and 418 and 1616 mg/kg-day, respectively for animals exposed for 14 days. A positive control group (for liver enzyme induction and decreased circulating thyroid levels) received 10 mL/kg body weight (80 mg/kg-day) phenobarbital (PB) by oral gavage daily.

TSH, T_3 and T_4 levels were determined by radioimmunoassay for all animals at sacrifice. Hepatic gene transcription levels were determined for 1) Cyp1a1, 2) the constitutive androstane receptor (CAR)-responsive elements Cyp2b9, Cyp2b10, UGT1a1, UGT2b5 and Sult 2a2, 3) the pregnane-X-receptor mediated Cyp3a11, and 4) the sulfotransferase Sult1a1 by quantitative polymerase chain reaction.

Cyp1A-dependent 7-ethoxyresorufin-O-deethylase (EROD), Cyp3A-dependent 7-benzoxyresorufin-O-debenzylase (BROD), Cyp2B-dependent 7-pentoxyresorufin-O-dealkylase (PROD), lauric acid hydroxylation, and uridine diphosphate glucuronyl transferase activities were assayed in liver microsomes prepared from a pool of 3 mice/exposure group.

TBA did not induce treatment-related clinical signs or body weight changes in either exposure group. The authors reported visibly enlarged livers in 3 of 15 animals for both TBA exposure groups after 14 days but not 3 days of exposure. However, the authors also reported no effects on either absolute or relative liver weight in any TBA-exposed animals.

A histopathological examination was performed for the thyroids of all animals, and for the livers of 5 animals/group (6 animals for controls). TBA was not observed to induce mouse thyroid histopathological changes at the exposure levels used. A diffuse centrilobular hepatocellular hypertrophy similar to that observed with PB was observed in the 20 mg/mL exposure group but not the 2 mg/mL exposure group, which had a lower incidence and severity than was observed in the PB-treated animals. The hepatocellular hypertrophy data are listed in Table 13.

Table 13. Centrilobular hepatocellular hypertrophy incidence and severity data from B6C3F$_1$ mice treated with *tertiary*-butanol (TBA) or phenobarbital (PB) (Blanck *et al*, 2010).

Treatment	TBA (mg/mL)			PB (mg/kg-day)
	0	**2**	**20**	**80**
# animals	6	5	5	6
Diffuse centrilobular hepatocellular hypertrophy				
Minimal (grade 1)	0	0	1	4
Slight (grade 2)	0	0	1	1
Total	0	0	2	5

PB significantly increased gene transcription of all the genes surveyed except Cyp2b9 and Ugt2b5 at both 3 and 14 days of exposure. TBA did not increase transcription of any of the genes surveyed after 3 days of exposure. After 14 days of TBA exposure, significant gene transcription increases were noted for Cyp2b10 and Sult1a1 in both exposure groups, and for Cyp2b9 in the 20 mg/mL exposure group.

PB significantly increased cytochrome P-450 content and EROD, PROD and BROD activity after both 3 and 14 days of exposure. TBA significantly increased BROD activity in both exposure groups, and significantly increased P-450 content, PROD and EROD activity in the 20 mg/mL exposure group but not the 2 mg/mL exposure group after 14 days of treatment.

TBA (both exposure groups) and PB did not produce a statistically significant increase in TSH levels compared to controls after either 3 or 14 days exposure. T_3 levels were not significantly reduced compared to controls by either TBA (both

exposure groups) or PB after 3 days of exposure. TBA caused a small but statistically significant decrease in T_3 levels after 14 days of exposure (12% and 13% decrease in the 2 and 20 mg/mL exposure groups, respectively; $p < 0.01$ for both groups). PB-induced reductions in T_3 levels after 14 days of exposure were greater than those observed for TBA (21%, $p < 0.01$). T_4 levels were also not significantly reduced compared to controls by TBA (both exposure groups) after 3 days of exposure. A moderate statistically significant reduction in T_4 levels was noted after 14 days of TBA exposure in both the 2 and 20 mg/mL exposure groups (15% and 22% reduction, respectively; $p < 0.01$). In contrast, PB significantly reduced T_4 levels in the animals after both 3 and 14 days of exposure (34% and 48% reduction, respectively; $p < 0.01$), and produced a greater depression of T_4 levels than TBA in this study.

Genotoxicity

Both TBAc and TBA have been tested in several genotoxicity assays. A tabular summary of the results of that testing is listed in Table 14.

Table 14. Genotoxicity testing summary for *tertiary*-butyl acetate and *tertiary*-butanol.

Assay	Cell type or species/strain	Metabolic Activation		Reference
		without	with	
***tertiary*-butyl acetate**				
Bacterial gene mutation	*Salmonella* typhimurium TA 98, TA100, TA1535, TA1537, TA102	-	-	Huntingdon Life Sciences Ltd. (2000a)
	Escherichia coli, strain WP2uvrA/pKM 101 (CM891)	-	-	Huntingdon Life Sciences Ltd. (2000a)
Chromosomal aberrations	*in vitro* human lymphocytes	-	-	Cruzan and Kirkpatrick (2006)
Rat micronucleus assay	Male/female Sprague-Dawley rats	NA	-	Cruzan and Kirkpatrick (2006)
***tertiary*-butanol**				
DNA damage, Comet assay	human HL-60 leukemia cells	+	NA	Tang *et al*. (1997)
	Rat-1 diploid rat fibroblast cells	+	NT	Sgambato *et al.* (2009)
8-OHdG DNA adducts (oxidative DNA damage)	Rat-1 diploid rat fibroblast cells	+	NT	Sgambato *et al.* (2009)
DNA adducts	Male Kunming (outbred) mice	NA	+/-	Yuan *et al.* (2007)
Bacterial gene mutation	*S*. typhimurium TA98, TA100, TA1537, TA1538	-	-	NTP (1995)
Bacterial gene mutation	*S*. typhimurium TA102	NT	+	Williams-Hill *et al*. (1999)
	S. typhimurium TA102	-	-	Huntingdon Life Sciences Ltd. (2000a)
Bacterial gene mutation	*S*. typhimurium TA102	-	-	McGregor *et al*. (2005)
Mammalian gene mutation	L5178Y mouse lymphoma cells	+/-	-	NTP (1995)

Assay	Cell type or species/strain	Metabolic Activation		Reference
		without	with	
Chromosomal aberrations	Chinese hamster ovary (CHO) cells	-	-	NTP (1995)
Micronucleus induction	Male/female B6C3F$_1$ mice	NA	-	NTP (1995)

Abbreviations: +/-: equivocal. +: positive. -: negative. NA: not applicable. NT: not tested. 8-OHdG: 8-hydroxydeoxyguanosine.

Tertiary-Butyl Acetate

Bacterial mutation tests

Huntingdon Life Sciences Ltd. (2000a) evaluated the genotoxicity of TBAc in bacteria, using the *Salmonella* typhimurium strains TA1535, TA1537, TA98, TA100 and TA 102, and a tryptophan dependent mutant of *Escherichia coli*, strain WP2uvrA/pKM 101 (CM891). Test chemicals were dissolved in dimethyl sulphoxide (DMSO), which was also used as a negative control. Appropriate positive controls were included in the assay procedure. Duplicate assays were performed in the presence and absence of Aroclor 1254-induced rat liver S9. The test strains were pre-incubated with TBAc in gas-tight glass vials (to minimize loss of volatile test substance) prior to plating out. Test concentrations of TBAc used were 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in an initial range finding assay, and 50, 150, 500, 1500 and 5000 µg/plate in the final assay.

Test strain toxicity was observed after TBAc exposure at 5000 µg/plate. TBAc was not mutagenic in any bacterial test strain in the presence or absence of rat liver S9.

Human lymphocyte chromosomal damage in vitro

Cruzan and Kirkpatrick (2006) examined the ability of TBAc to cause chromosomal aberrations in human lymphocytes exposed *in vitro*. Human lymphocytes, in whole blood culture, were stimulated to divide by addition of phytohaemagglutinin, and exposed to the test substance both in the presence and absence of rat liver S9 mix. Solvent and positive control cultures were also prepared. Two hours before the end of the incubation period, cell division was arrested using colcemid and the cells harvested and slides prepared, so that metaphase cells could be examined for chromosomal damage.

In order to assess the toxicity of TBAc to cultured human lymphocytes, the mitotic index was calculated for all cultures treated with the test substance and the solvent control. On the basis of these data, the following concentrations were selected for metaphase analysis:

First test:
Without and with S9 mix -
3 hours treatment, 18 hours recovery: 290, 580 and 1160 µg/mL.

Second test:
Without S9 mix - 21 hours continuous treatment: 290, 580 and 1160 µg/mL.
With S9 mix - 3 hours treatment, 18 hours recovery: 290, 580 and 1160 µg/mL.

The maximum test concentration, 1160 µg/mL, corresponded to a 10 mM solution of TBAc.

TBAc caused no significant increase in the proportion of metaphase figures containing chromosomal aberrations (excluding gap damage) at any dose level when compared with the solvent control, in either test in both the absence and presence of S9 mix. A quantitative analysis for polyploidy was made in cultures treated with the negative control and highest dose level. No statistically significant increases in the proportion of polyploid cells were seen. The authors concluded that TBAc did not induce chromosomal aberration in human lymphocytes exposed *in vitro*.

Rat micronucleus assay

The ability of TBAc to induce micronuclei (indicative of chromosomal damage) in the bone marrow cells of rat exposed to TBAc by inhalation was studied by Cruzan and Kirkpatrick (2006). Male and female Sprague-Dawley rats were subjected to a single 6-hour nose-only inhalation exposure to TBAc at exposure levels of 100, 400 and 1600 ppm (5 animals/sex/100 and 400 ppm groups; 10 animals/sex/1600 ppm group). A negative control group received clean air only (10 animals/sex/group). A positive control group was dosed by gavage with cyclophosphamide at 20 mg/kg bodyweight.

Bone marrow smears were obtained from five male and five female animals in the negative control group, each of the test substance groups and the positive control group 24 hours after treatment. In addition, bone marrow smears were obtained from five male and five female animals in the negative control and high dose treatment groups 48 hours after treatment. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. The proportion of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. The incidence of micronucleated mature erythrocytes was also evaluated.

No significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the proportion of immature erythrocytes were observed in rats treated with TBAc and sacrificed 24 or 48 hours later, compared to vehicle control values.

The authors concluded that TBAc did not show any evidence of causing chromosome damage or bone marrow cell toxicity in male or female rats exposed to TBAc by nose-only inhalation exposure.

Tertiary-Butanol

DNA damage

Tang *et al*. (1997) examined the ability of TBA to induce DNA damage in human leukemia HL-60 cells using a Comet assay (single cell gel electrophoresis). The results reported were the mean of three replicate experiments. However, the authors did not report cross-experiment variation. TBA was not significantly cytotoxic at any of the concentrations tested (1, 5, 10, and 30 mmol/L; 74, 371, 741, and 2224 µg/mL) as determined by lactate dehydrogenase (LDH) release. However, both the number of cells demonstrating DNA damage and the severity of damage increased significantly in a dose–responsive manner at all concentrations tested.

Yuan *et al.* (2007) used an accelerator mass spectrometry assay to study DNA adducts induced in mice by ^{14}C-radiolabeled TBA. Male Kunming (outbred) mice (6/group) were exposed to 0, 0.099, 0.99, 10, 101, or 997 µg ^{14}C-TBA/kg body weight dissolved in saline, using a dosing volume of 8 mL/kg body weight. Animals were sacrificed at six hours post-treatment and the liver, kidneys, and lungs were collected for DNA isolation. Extracted DNA samples from the organs listed above were then analyzed for DNA adducts using an accelerator mass spectrometer. A dose-dependent increase in ^{14}C-TBA-DNA adducts was reported for liver, kidney and lung.

Acetone has been identified as a minor metabolite of TBA in rats (OEHHA has not identified any TBA metabolism studies in mice). Acetone carbon can be incorporated into glucose, which can participate in the pentose phosphate pathway, producing ribose-5-phosphate, a precursor of deoxyribose. The identity of the DNA adducts was not compared and confirmed using synthetic standards in the study, which makes this evaluation inconclusive.

The effects of TBA on a diploid rat fibroblast cell line (Rat-1) were studied by Sgambato *et al.* (2009). Exposure of Rat-1 cells to 0.44 mM (33 µg/mL) TBA for 4 hours caused a significant increase (225%; $p < 0.01$) in 8-hydroxydeoxyguanosine (8-OHdG) levels as measured by an immunohistochemistry assay using an anti-8-OHdG antibody. 8-OHdG is generally considered to be a marker of DNA oxidative damage.

TBA was also evaluated for induction of DNA damage in Rat-1 cells in the comet assay. Rat-1 cells were exposed to 0.44 mM (33 µg/mL) TBA for 4 hours, and the cells were assayed for DNA damage at 30 minutes, 4 hours and 12 hours. DNA damage peaked at 30 minutes, as demonstrated by increases in tail length, percent migration and tail moment, and then declined at 4 and 12 hours.

Cytotoxicity is a potential confounder in interpreting the results of the Sgambato *et al.* (2009) comet assay data. The study employed a single 48-hour IC50 (causing 50% viability of treated cells) test concentration. The IC50 was determined using data from a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The MTT assay measures NADPH-dependent cellular oxidoreductase enzyme

activity, and can produce positive results if either a cytotoxic or cytostatic effect is occurring. This test concentration exceeded the upper limit concentration (IC30) recommended for such tests to avoid potential confounding by cytotoxicity (Tice *et al*., 2000).

However, the authors presented data indicating that TBA induced a block in cell cycle progression at the G0/G1 phase, which may have been caused by the observed increased expression of p27Kip1 and reduced expression of cyclin D1. The authors also stated that "It is noteworthy that no significant increase in the number of dead detached cells was observed in cell cultures after exposure to the two compounds, thus suggesting that the observed reduction in cell number was mainly due to a cytostatic rather than to a cytotoxic effect (data not shown)." The degree to which cytotoxicity may have been a confounder in interpreting the results of the comet assay data described above is unclear.

Bacterial mutation assays

NTP (1995) found TBA to be negative in the *Salmonella*/mammalian microsomal mutation assay in the presence and absence of Aroclor 1254-induced rat or hamster liver S9 in test strains TA98, TA100, TA1537 and TA1538 at concentrations of 100, 333, 1000, 3333 and 10,000 µg/plate.

The genotoxicity of TBA was assayed using the standard *Salmonella*/mammalian microsomal mutation assay with TA102, a strain that detects oxidative DNA damage and contains a functional *uvr*B gene, rendering it excision repair proficient (Williams-Hill *et al*., 1999). The assay was performed in the presence of Aroclor-induced rat liver S-9, using concentrations ranging from approximately 750 to 3750 µg/plate. TBA caused a maximum of 800 revertants/plate (approximately 2-fold greater than control) at a concentration of approximately 2500 µg/plate. The authors concluded that TBA induces a mutagenic pathway involving oxidation of DNA bases and an intact repair system.

Huntingdon Life Sciences Ltd. (2000a) evaluated the genotoxicity of TBA concurrently with TBAc in *Salmonella* strain TA102 only, employing the same methodology as used for TBAc. Duplicate assays were performed in the presence and absence of Aroclor 1254-induced rat liver S9. The test strains were pre-incubated with TBA in gas-tight glass vials (to minimize loss of volatile test substance) prior to plating out. Test concentrations of TBA used were 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in an initial range finding assay, and 50, 150, 500, 1500 and 5000 µg/plate in the final assay. No toxicity was observed after TBA exposure. TBA was not genotoxic to *Salmonella* strain TA102 in the presence or absence of rat liver S9.

McGregor *et al*. (2005) tested TBA for mutagenicity in S. typhimurium (test strain TA102) in the presence and absence of Aroclor 1254 induced rat liver S9 in two separate experiments. TBA was dissolved in either DMSO or water at concentrations of 0, 100, 200, 500, 1000, 2500 and 5000 µg/plate. TBA dissolved in water in the

absence of rat liver S9 caused a small increase in mutagenicity in the two experiments (maximum response 1.5-fold and 1.7-fold greater than control in experiments 1 and 2, respectively), but the increase was less than the 2-fold increase that is usually considered significant.

Mammalian gene mutation assays

TBA did not induce mutations in the presence of Aroclor 1254-induced rat liver S9 in the L5178Y mouse lymphoma mutation assay in two trials at concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL (NTP, 1995). TBA did cause a significant increase in mutations in the absence of S9 in one trial at the 5000 µg/mL concentration. The number of mutations induced increased with dose, but NTP did not present trend test data. A second TBA trial in the absence of S9 was negative, but NTP did not present the results from that trial because it did not meet quality control standards for that assay. NTP (1995) concluded that TBA did not induce mutations in the L5178Y mouse lymphoma mutation assay.

Mammalian chromosomal damage assays

TBA did not induce chromosomal aberrations *in vitro* in Chinese hamster ovary (CHO) cells in the presence and absence of Aroclor 1254-induced rat liver S9 at concentrations ranging from 160 to 5000 µg/mL (NTP, 1995). TBA also did not induce sister chromatid exchanges (SCEs) in the presence of Aroclor 1254-induced rat liver S9 in CHO cells. TBA did cause a significant increase in SCEs in the absence of S9 in one trial at the 5000 µg/mL concentration. The number of SCEs induced increased with dose, but NTP did not present trend test data. A second TBA trial in the absence of S9 was negative. NTP (1995) concluded that TBA did not induce SCEs in CHO cells.

Additionally, TBA did not induce micronuclei (indicative of chromosomal damage) in normochromatic erythrocytes obtained from male and female $B6C3F_1$ mice exposed to 0, 3000, 5000, 10,000, 20,000 or 40,000 ppm TBA in drinking water for 13 weeks (NTP, 1995).

V. CANCER HAZARD EVALUATION

Male rat kidney tumor data

TBAc is metabolized in rats to TBA (Groth and Freundt, 1994; Huntingdon Life Sciences Ltd., 2000b). Oral TBA exposure induces renal tumors in male rats and thyroid follicular cell tumors in female mice (NTP, 1995). The possibility that the male rat kidney tumors observed after oral TBA exposure may be related to a TBA-α2u interaction resulting in α2u nephropathy has been raised by Borghoff *et al.* (2001), McGregor and Hard (2001), and Hard *et al.* (2011). The defining feature of α2u nephropathy is the rapid accumulation of protein (or hyaline) droplets in the renal proximal tubule cells of many strains of male rats following chemical treatment. Female rats do not appear to produce α2u. α2u is usually degraded in the lysosomal compartment of the renal proximal tubule cells. It is believed that binding of α2u to

chemicals results in an impairment of the lysosomal degradation of this protein, leading to an accumulation of protein droplets containing α2u. Protein droplet accumulation occurs rapidly, and leads to progressive renal injury, characterized by single cell degeneration and necrosis in the renal proximal tubule. Renal proximal tubule necrosis causes compensatory cell proliferation in the renal cortex. Cessation of cell proliferation occurs shortly after stopping acute exposures to α2u-inducers, and a restoration of normal renal architecture can occur. Chronic exposure to α2u-inducers leads to linear papillary mineralization. Atypical hyperplastic cellular foci may eventually be observed in the proximal tubules, and these foci may eventually progress to renal adenomas and carcinomas (Swenberg and Lehman-McKeeman, 1999).

The International Agency for Research on Cancer (IARC) (1999), developed criteria on whether chemicals induce male rat kidney tumors through increased accumulation of α2u-globulins. When these criteria are fully met, the chemicals are not expected to cause cancer in humans through that mechanism.

The criteria for an agent causing kidney tumors through an α2u-globulin-associated response in male rats (IARC, 1999) are:

1. Lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data

2. Male rat specificity for nephropathy and renal tumorigenicity

3. Induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory

4. Identification of the protein accumulating in tubule cells as α2u-globulin

5. Reversible binding of the chemical or metabolite to α2u-globulin

6. Induction of sustained increased cell proliferation in the renal cortex

7. Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets, α2u-globulin accumulation, cell proliferation).

OEHHA believes that TBA toxicity fits criteria 3, 4 and 5, partly fits criteria 2 and 6, but does not fit criteria 1 and 7 (Table 15). Thus, the increased renal tumor incidences observed by NTP (1995) in TBA-exposed male rats should be considered appropriate for use in human cancer risk assessment. A detailed explanation of the relationship between the TBA toxicity data and the IARC α2u criteria is provided below.

Table 15. OEHHA evaluation of whether *tertiary*-butanol (TBA) data fit the α2u-globulin mode of action criteria set by the International Agency for Research on Cancer (IARC, 1999).

IARC Criteria for an α2u-Globulin Mode of Action	Do TBA data meet this criterion?
1. Non-genotoxicity	No
2. Male rat specificity	Partly
3. Characteristic histopathological changes	Yes
4. Identification of α2u globulin in renal tubule cells	Yes
5. Reversible TBA binding to α2u globulin	Yes
6. Sustained increased renal cortex cell proliferation	Partly
7. Dose-response similarities between tumors and histopathological endpoints	No

TBA toxicity fits some of these criteria:

Criterion 3:

Acute and subchronic TBA exposure has been shown to exacerbate hyaline droplet formation in male rats (Takahashi *et al.*, 1993; NTP, 1995; Borghoff *et al.*, 2001), and chronic histopathological changes including linear papillary mineralization have been observed in TBA-exposed male rats (NTP, 1995).

Criterion 4:

Immunohistochemical staining of protein droplets for α2u in control and TBA-exposed male rat kidney sections within renal proximal tubules indicated α2u incorporation (Borghoff *et al.*, 2001). Additionally, Faber *et al*. (2014) noted consistent renal α2u-globulin accumulation in male rat 13-week TBAc exposure groups as measured by both immunohistopathology and ELISA analysis.

Criterion 5:

Reversible binding of TBA to α2u has been demonstrated (Williams and Borghoff, 2001), although a binding affinity was not determined.

TBA partially fits several of the other criteria listed above:

Criterion 2:

The NTP TBA 2-year study (1995) demonstrated renal tumors only in male rats.

However, adverse renal effects associated with TBA exposure have been observed in female rats. NTP (1995) reported nephropathy in both male and female rats in

both the 13-week and 2-year oral studies. In the 13-week study, renal lesion severity did not increase in the exposed female rats, but lesion incidence increased significantly compared to controls in the 10, 20 and 40 mg/mL dose groups. In the 2-year study, NTP reported nephropathy in all male and female rats at the 15-month interim sacrifice, with severity scores ranging from minimal to mild. Almost all female rats exhibited nephropathy at study termination, and severity scores demonstrated a treatment-related increase (Table 16).

Table 16. Incidences (and severity grades) of nonneoplastic lesions of the kidney in male and female rats in the 2-year drinking water study of *tertiary*-butanol (NTP, 1995).

Male Rats	**0 mg/mL**	**1.25 mg/mL**	**2.5 mg/mL**	**5 mg/mL**
Kidney[a]	50	50	50	50
Cyst[b]	0	0	0	1
Hemorrhage	0	0	0	1
Hydronephrosis	0	1	0	1
Hyperplasia, lymphoid	0	0	0	1
Inflammation, Suppurative	10	18	12	9
Mineralization	26	28	35	48
Nephropathy	49	49	50	50
Thrombosis	0	0	0	1
Interstitial Tissue, Pigmentation	0	0	0	1
Renal Tubule, Cytoplasmic Alteration	0	0	1	0
Renal Tubule, Hyperplasia	3 (1.7)	7 (1.7)	6 (2.0)	6 (1.7)
Renal Tubule, Hyperplasia, Oncocytic	0	0	2	0
Renal Tubule, Pigmentation	7	9	6	12
Transitional Epithelium, Hyperplasia	25	32	36	40
Female Rats	**0 mg/mL**	**2.5 mg/mL**	**5 mg/mL**	**10 mg/mL**
Kidney	50	50	50	50
Inflammation, Suppurative	2 (1.0)	3 (1.3)	13** (1.0)	17** (1.1)
Mineralization	49 (2.6)	50 (2.6)	50 (2.7)	50 (2.9)
Nephropathy	48 (1.6)	47 (1.9)*	48 (2.3)**	50 (2.9)**
Renal Tubule, Hyperplasia	0	0	0	1 (1.0)
Transitional Epithelium, Hyperplasia	0	0	3 (1.0)	17** (1.4)

Table adapted from NTP (1995).
*Significantly different ($p \leq 0.05$) from the control group by the logistic regression (incidences) or by the Mann-Whitney U test (severity grades).
**($p \leq 0.01$)
[a]Number of animals with kidney examined microscopically.
[b]Number of animals with lesion
Numbers in parentheses represent the average severity of lesions in affected animals:
1 = minimal; 2 = mild; 3 = moderate; 4 = marked

The NTP described the renal lesions as “thickened tubule and glomerular basement membranes, basophilic foci of regenerating tubule epithelium, intratubule protein casts, focal mononuclear inflammatory cell aggregates within areas of interstitial fibrosis and scarring, and glomerular sclerosis.” This description is consistent with the US Environmental Protection Agency’s (US EPA’s) description of chronic progressive nephropathy (CPN) (1991). CPN is a common spontaneous lesion in rats, is believed to be related to age, and is not necessarily the result of chemical toxicity (US EPA, 1991). However, CPN can be exacerbated by chemical treatment (NTP, 1997), and such exacerbation can be included in support of a chemical hazard evaluation.

Additionally, an increase in the incidence of renal suppurative inflammation and transitional epithelium hyperplasia (TEH) was noted in female rats in the 2-year oral TBA study (NTP, 1995). These lesions were considered by NTP to be related to nephropathy, but the lesions have different dose-response relationships than those reported for CPN.

Male rat renal suppurative inflammation was substantial in the control animals (20% incidence) and did not demonstrate a dose-response relationship with treatment. In contrast, female rat renal suppurative inflammation incidence was low in controls (4%), and demonstrated a treatment-related incidence increase (Table 16). In contrast, suppurative inflammation was not reported in male or female rats in the 13-week oral study (NTP, 1995) or in the 13-week inhalation study (NTP, 1997), while nephropathy was observed. This indicates that suppurative inflammation is not related to nephropathy and is treatment related.

Both male and female rats manifested renal TEH in the 2-year oral study. Control males demonstrated a high incidence of TEH (50%), and a positive dose-response with treatment was noted for both incidence and severity. However, while increases in TEH incidence and severity were noted for mid- and high-dose females, TEH was not observed in control and low-dose females. Hard *et al.* (2011) evaluated the NTP 1995 male and female rat renal histopathology slides and concluded from a visual evaluation of those slides that the TEH noted in the high-dose female rats was the result of advanced CPN and was not treatment related. However, Hard *et al.* (2011) did not perform a comparative evaluation of the nephropathy and TEH dose-response as part of their evaluation. As with suppurative inflammation, the contrasting dose-responses for TEH and nephropathy indicate that TEH is not related to nephropathy and is treatment related.

Overall, these observations do not fit Criterion 2. Melnick *et al*. (1997) noted that this nephrotoxic response in the female rat suggests the possibility of other processes leading to or influencing the kidney tumor response.

Criterion 6:

The data on the sustained induction of renal tubular epithelial cell proliferation in male rats by TBA is mixed. A dose-dependent increase in cell proliferation in the

renal tubular epithelial cells of male rats exposed to TBA by inhalation was observed by Borghoff *et al.* (2001) at all TBA concentrations tested (250, 450 and 1750 ppm). The animals in this study were only exposed for 10 days, so this study did not provide information on whether cell proliferation was sustained chronically.

Lindamood *et al.* (1992) presented selected data from the NTP (1995) 90-day drinking water study of TBA in male and female F344 rats at concentrations of 0, 0.25, 0.5, 1, 2 and 4% (0, 2.5, 5, 10, 20 and 40 mg/mL). The data included male rat renal tubular epithelial cell proliferation as measured by PCNA incorporation. Female rat data was not presented. Median PCNA scores were increased in the 1% (p = 0.0511) and 2% dose groups (p = 0.0215), but not the 4% dose group.

Takahashi *et al.* (1993) presented an analysis of renal cortical sections from the NTP (1995) 13-week TBA drinking water exposure study which included PCNA incorporation assay data for male rat renal tubular epithelial cell proliferation. A statistically significant ($p < 0.05$) increase in median (not mean) cell proliferation was only noted in the second highest exposure group at 20 mg/mL.

More recent data by Faber *et al.* (2014) indicated that TBAc exposure did not cause increases in rat kidney tubular cell proliferation after 13 weeks of TBAc exposure at concentrations up to 1600 ppm (2160 mg/kg-day) by inhalation as measured by PCNA assessment.

<u>TBA toxicity does not fit the following criteria:</u>

Criterion 1:

Positive genotoxicity data exist for TBA. TBA has been reported to cause DNA damage in human leukemia HL-60 cells using a Comet assay (Tang *et al*., 1997), and induce 8-OHdG formation and DNA damage measured via the Comet assay in Rat-1 cells (Sgambato *et al*., 2009). Additionally, TBA has been demonstrated to induce mutations in a *Salmonella* strain known to be sensitive to oxidative DNA damage (TA102) in the presence of rat liver S9 (Williams-Hill *et al.*, 1999). These data indicate that TBA has genotoxic potential, and precludes a determination of non-genotoxicity.

Criterion 7:

While α2u staining was slightly greater in male rats exposed to TBA as compared to control male rats (Criterion 3) (Borghoff *et al*., 2001), the authors stated that no TBA exposure-related increase in α2u staining intensity in male rats was noted. A significant increase in renal cytosol α2u concentrations as determined by an ELISA assay was noted in the male rat 1750 ppm group ($p < 0.05$) compared to controls, in contrast to the α2u staining evaluation, where no TBA exposure-related α2u increase was noted. However, very little increase, if any, in α2u concentrations was noted in the 250 and 450 ppm groups as determined by an ELISA assay. The renal α2u concentration in the 1750 ppm group compared to controls also appears to be substantially less than that observed for the α2u inducer TMP (Prescott-Matthews *et*

al., 1997). The authors speculated that the mild increase in TBA-exposed male rat α2u concentrations might only be detectable using an ELISA assay due to the greater sensitivity of the method compared to immunohistochemical staining.

Additionally, Faber *et al.* (2014) stated that renal α2u-globulin accumulation was consistent throughout male rat 13-week TBAc exposure groups as measured by both immunohistopathology and ELISA analysis. The authors did not present a statistical analysis of these data. However, a graphical depiction of male rat kidney α2u-globulin levels appeared to show that there was probably no significant difference between the TBAc exposure groups, and possibly no significant difference between some of the TBAc exposure groups and the control group, suggesting a weak dose-response relationship between TBAc exposure and renal α2u-globulin accumulation in this study. Since TBAc is quickly and extensively metabolized to TBA in rats, this study is relevant to a TBA-induced male rat kidney tumor mode of action consideration.

The dose response relationship between hyaline droplet severity and renal tumor incidence was not particularly strong in the NTP 1995 study. Male rats exposed to TBA for 13 weeks developed increased hyaline droplet accumulation within renal tubule epithelium and lumens at all doses of TBA, but the droplet accumulation was minimal at the dose demonstrating a significantly increased tumor incidence (2.5 mg/mL). Linear mineralization (associated with α2u induction) incidence increased with dose in the NTP 2-year study (1995), but severity scores did not exhibit a dose-response. NTP also observed that there was no morphologic evidence of extensive cell necrosis (granular cast formation) resulting from TBA exposure.

In addition, male rats in the TBA inhalation study by Borghoff *et al.* (2001) did not demonstrate significant increases in either hyaline droplet accumulation or renal α2u concentrations at estimated doses approximating those resulting in increased renal tumors in the 1995 NTP study.

Lindamood *et al.* (1992) and Takahashi *et al.* (1993) presented an analysis of renal cortical sections from the NTP (1995) 13-week TBA drinking water exposure study which included an evaluation of male rat renal tubular epithelial cell proliferation as measured by PCNA-incorporation. A statistically significant ($p < 0.05$) increase in median (not mean) cell proliferation was noted in the 20 mg/mL exposure group, with a borderline significant increase ($p = 0.0511$) noted for the 10 mg/mL exposure group. This is well above the exposure level (2.5 mg/mL) which induced male rat renal tubule tumors in the NTP (1995) 2-year TBA drinking water exposure study.

The hypothetical progression of events leading from α2u nephropathy to renal tumors is that an accumulation of hyaline droplets containing α2u in the renal proximal tubules results in cell death, then compensatory cell proliferation, which leads to increased renal tumors. Swenberg and Lehman-McKeeman (1999) note that “Dose related and male rat specific increases in cell proliferation have been demonstrated with all of the [α2u-inducing] chemicals evaluated, and the dose response relationships for cell proliferation parallel those for hyaline droplet formation and the

induction of renal tumours." However, in the case of TBA, male rat kidney tumor induction takes place at exposure levels where cell proliferation and hyaline droplet formation are not substantially increased.

The above data do not rule out the possibility of increased renal α2u concentrations playing a role in the increased renal tumor incidence seen in TBA-exposed male rats, either directly through the induction of nephropathy, or indirectly through increasing the TBA concentration in the kidney. However, Doi *et al.* (2007) evaluated several known renal α2u nephropathy-inducers for relationships between events resulting from induction of α2u (renal *α*2u-globulin concentrations, cell turnover indices, histopathological evidence of *α*2u-associated nephropathy) in 90-day studies and renal tumors in 2-year studies. Doi *et al.* (2007) found "no or at best weak associations of tumor responses with renal *α*2u-globulin concentrations, indices of cell turnover, or microscopic evidence of *α*2u-associated nephropathy" for renal *α*2u-globulin inducers in general.

As described above, 1) the dose response relationship between hyaline droplet severity, cell proliferation and renal tumor incidence in TBA-exposed rats is weak; 2) TBA exposure has been demonstrated to cause adverse renal effects (nephropathy, suppurative inflammation, transitional epithelial hyperplasia) in female rats; 3) positive TBA genotoxicity data exists. These data indicate that it would not be appropriate to determine that the increased renal tumors observed in TBA-exposed male rats are solely due to α2u-induced nephropathy.

Hard *et al.* (2011) also stated that both α2u-induced nephropathy and exacerbation of CPN were responsible for induction of renal tumors in TBA-treated male rats. Doi *et al.* (2007) did note that CPN severity was at least somewhat predictive for tumor induction in three of the four α2u-inducers studied (limonene, decalin and propylene glycol mono-*t*-butyl ether but not Stoddard solvent IIc). However, Melnick *et al.* (2012) evaluated 58 NTP carcinogenicity studies using male F344 rats and 11 studies using female F344 rats for relationships between exacerbated CPN and induction of rat renal tumors. Melnick *et al.* (2012) found widespread inconsistencies in the hypothesized relationship, and stated that "Because the proposed hypothesis lacks evidence of biological plausibility, and due to inconsistent relationships between exacerbated CPN and kidney tumor incidence in carcinogenicity studies in rats, dismissing the human relevance of kidney tumors induced by chemicals that also exacerbate CPN in rats would be wrong."

Thus, the increased renal tumor incidences observed by NTP (1995) in TBA-exposed male rats should be considered to be suitable for use in human cancer risk assessment.

Female mouse thyroid follicular cell tumor data

Anti-thyroid cancer Mode of Action (MOA) data

McClain (2001) prepared an expert opinion for Lyondell Chemical Co. regarding the increased thyroid follicular cell tumor incidence observed by NTP (1995) in female TBA-exposed mice, which stated that "The thyroid gland hyperplasia noted in male and female mice and the increased follicular cell tumor incidence in female mice are consistent with an epigenetic mode of action. It is postulated that the thyroid gland hyperplasia and neoplasia are the consequence of microsomal enzyme induction of thyroid hormone metabolism. This results in a compensatory increase in pituitary TSH which, with long term, low level stimulation of the thyroid gland results in follicular cell hypertrophy, hyperplasia and ultimately neoplasia in rodents."

It has been suggested that thyroid hormone hepatic metabolism and excretion may lead to compensation by the thyroid gland for this increased excretion, potentially resulting in stimulation of the hypothalamic-pituitary-thyroid axis and possibly secondary hyperplastic or neoplastic changes in the thyroid (Curran and de Groot, 1991).

This opinion did not state whether the female mouse thyroid tumor data were not applicable to human cancer risk assessment. However, some have taken the position that "the available evidence indicates that the thyroid tumors observed in the high dose female mice are due to a threshold mechanism - one to which humans are much less susceptible than rodents. Since the high dose of TBA was 2110 mg/kg/day, and yet higher doses of TBAc would be required to achieve such TBA levels (see next section), there is very little likelihood that humans could experience exposures sufficient to cause such tumors (Lyondell Chemical Co., 2001)."

However, the available mouse data does not support the expert opinion for Lyondell Chemical Co. that a threshold mechanism is the cause for TBA carcinogenicity in that species.

Liver weights in male and female mice exposed orally to TBA for 13 weeks (NTP, 1995) were only slightly increased in the 20 and 40 mg/mL male and 40 mg/mL female groups (115% and 107% of control for the male 20 and 40 mg/mL groups, respectively; 113% of control for the female 40 mg/mL group). Hepatic enzyme induction is often associated with increases in liver weight (Maronpot *et al.*, 2010).

Blanck *et al*. (2010) did not observe increases in either absolute or relative (to body weight) liver weights in groups of 15 female B6C3F$_1$ mice exposed to concentrations of 0, 2, or 20 mg/mL TBA in drinking water for either 3 or 14 days. TBA significantly increased BROD activity in both exposure groups, and significantly increased P-450 content and PROD activity in the 20 mg/mL exposure group but not the 2 mg/mL exposure group after 14 days of treatment. The magnitude of increase observed after 14 days of exposure to 20 mg/mL TBA (about 1.5-, 2-, and 12-fold above

controls, for P-450, PROD and BROD activity, respectively) was substantially less than the increase induced by the liver enzyme inducer PB (2.2-, 8-, and 72-fold above controls, for P-450, PROD and BROD activity, respectively). However, Blanck *et al*. (2010) also found that TBA did not induce mouse thyroid histopathological changes, and did not produce a statistically significant increase in TSH levels compared to controls after either 3 or 14 days exposure. TBA caused a small decrease in T_3 levels and a moderate decrease in T_4 levels after 14 days of exposure. PB-induced decreases in T_3 and T_4 levels in this study were substantially greater than those produced by TBA.

Faber *et al.* (2014) did not observe significant thyroid gland histopathological changes or alterations in thyroid/parathyroid gland weights in TBAc-exposed mice compared to controls after 13 weeks of exposure. Additionally, no significant increases in TSH levels or decreases in T_3 levels were observed in TBAc-exposed male or female mice compared to controls. Moderately decreased T_4 levels (32% reduction compared to controls) were only noted in the male 1600 ppm mouse exposure group. TBAc-exposed female mice demonstrated no change in T_4 levels compared to controls.

The above data on the effects of TBA in mice indicate that TBA: 1) causes little or no increases in absolute or relative liver weights; 2) does not induce cytochrome P450 activity to the same degree as seen with PB; 3) does not cause large decreases in T_3 or T_4 levels; 4) does not increase TSH levels; 5) does not cause acute abnormal mouse thyroid histopathological changes. Additionally, TBAc has not been observed to cause significant thyroid gland histopathological changes or alterations in thyroid/parathyroid gland weights in mice. TBAc has been observed to induce moderate decreases in T_4 levels at relatively high dose exposures in male but not female mice, but did not cause increases in TSH levels or decreases in T_3 levels in male or female mice.

Differential mouse sensitivity by sex to thyroid carcinogens

Data from the NTP (2017b) Organ Sites with Neoplasia database indicates that the number of NTP test chemicals associated with site-specific neoplasia that produced positive, clear or some evidence of thyroid follicular cell tumors was approximately equivalent for male and female mice. This suggests that female mice are not generically more sensitive than male mice to thyroid carcinogens.

TBAc inhalation concentration / NTP (1995) TBA dose level comparisons

Cruzan and Kirkpatrick (2006) and Bus *et al.* (2015) claim that TBAc toxicity limits the potential ability of achieving TBAc tumorigenic doses in mice. Inhalation of 3000 ppm TBAc for 6 hours caused prostration in mice (Cruzan and Kirkpatrick, 2006). TBA induced tumors in female mice given 2110 mg/kg-day in drinking water. Cruzan and Kirkpatrick back calculated the TBAc concentration required to produce a 2110 mg/kg-day TBA dose, assuming 50% metabolism of TBAc to TBA, and a 75% fractional absorption (FA). Cruzan and Kirkpatrick stated that 2110 mg/kg/day

equates to 6657 ppm (31,554 mg/m^3) TBAc, which exceeds the maximum tolerated dose (MTD) in mice.

However, the Cruzan and Kirkpatrick (2006) model overestimates the TBAc air concentrations required to produce an oral TBAc dose of 2115 mg/kg-day. In their algorithm, BW and minute volume (MV) are sensitive parameters. OEHHA recalculated the hypothetical TBAc inhalation concentration using the model published by Cruzan and Kirkpatrick (2006) for the 2110 mg/kg-day female mouse oral dose from the NTP (1995) study and found that concentration to be 2395 ppm (11,352 mg/m^3). This calculation was performed using an average BW for female mice (0.05 kg) in the NTP (1995) study, an MV calculated from US EPA guidance (2012a) that considers specific mouse BWs, and the FA and TBAc to TBA metabolic conversion factors (MCs) listed above (95% and 71%, respectively). The hypothetical TBAc concentration calculated by OEHHA (2393 ppm) is less than the 3000 ppm observed to cause acute CNS effects.

Additionally, as described above, Cruzan and Kirkpatrick (2006) reported a subacute study where male and female mice were exposed to TBAc inhalation concentrations as high as 1500 ppm (7584 mg/m^3) 6 hr/day for 14 days. Severe CNS symptoms were not reported for any dose group.

NTP (1997) performed both an 18-day and 13-week TBA inhalation study in male and female B6C3F$_1$ mice. In the 18-day study, the animals (5/group) were exposed to inhalation concentrations of 0, 450, 900, 1750, 3500 and 7000 ppm (0, 1387, 2774, 5394, 10,787 and 21,574 mg/m^3) for 6 hr/day, 5 days/week for 12 days, with a 6-day post-exposure observation period. All mice in the 7000 ppm (21,574 mg/m^3) group were moribund and subsequently sacrificed on study day 2. The 3500 ppm (10,787 mg/m^3) exposure group animals were prostrate following the first exposure through exposure day 3. Afterwards, clinical signs in the 3500 ppm (10,787 mg/m^3) group were primarily observed post exposure and included hypoactivity, ataxia and rapid respiration. NTP (1997) also noted hypoactivity, hyperactivity, and ataxia at lower incidences (specific incidences not stated). All female mice exposed to 3500 ppm (10,787 mg/m^3) TBA or less survived to the end of the study, and final exposed animal body weights were not significantly different from controls.

In the 13-week inhalation study, males and female mice (10/group) were exposed to TBA concentrations of 0, 135, 270, 540, 1080 and 2100 ppm (0, 416, 832, 1664, 3329 and 6472 mg/m^3). All female mice survived to study end, and final body weights were significantly decreased compared to controls in the 1080 ppm (3329 mg/m^3; 94% of controls; $p < 0.05$) and 2100 ppm (6472 mg/m^3; 92% of controls, $p < 0.01$) groups. No other clinical findings related to TBA treatment were reported. An MTD has been defined as “the highest dose of the test agent during the chronic study that can be predicted not to alter the animal’s normal longevity from effects other than carcinogenicity” (Sontag *et al*., 1976; Haseman and Lockhart, 1994). By this MTD definition, the 2100 ppm (6472 mg/m^3) TBA inhalation exposure did not exceed the MTD in female mice.

TBAc concentrations corresponding to those in the NTP (1997) 18-day study of TBA inhalation can be calculated assuming approximately equivalent absorption efficiency of TBAc and TBA and 71% metabolic conversion of TBAc to TBA (described above). The concentrations of TBAc that correspond to the NTP (1997) TBA 18-day inhalation study concentrations of 1750 and 3500 ppm (10,787 and 21,574 mg/m^3) are 2465 and 4925 ppm (11,684 and 23,345 mg/m^3), respectively (TBAc concentration = TBA concentration/0.71). The clinical effects noted for the TBA 1750 ppm (10,787 mg/m^3) exposure group, which corresponds to a TBAc 2465 ppm (11,684 mg/m^3) concentration, would not preclude the use of that concentration in a cancer bioassay. Similarly, the concentration of TBAc that corresponds to the NTP (1997) TBA 13-week inhalation study concentration of 2100 ppm (6472 mg/m^3) would be 2958 ppm (14,021 mg/m^3). No clinical effects were noted at that TBA concentration, suggesting that the corresponding TBAc concentration (2958 ppm; 14,021 mg/m^3) would be acceptable for use in a cancer bioassay.

In the NTP (1995) TBA drinking water study, female mice were not exposed to TBA levels greater than the MTD. Therefore, the female mouse thyroid tumor data should not be discounted on the basis of mortality in that study. Additionally, the information above suggests that the TBAc concentrations required to achieve the TBA dosing levels observed to induce thyroid follicular cell tumor in female mice in the 1995 NTP study would not exceed the MTD.

OEHHA carcinogen dose-response analysis methodology (OEHHA, 2009) assumes low dose linearity as a general default for carcinogens unless there is convincing evidence to the contrary. This results in an assumption that there is cancer risk at any carcinogen above zero. Also, there is a TBA dose (510 mg/kg-day) less than the high dose in the NTP (1995) study that causes an increased tumor incidence compared to controls. This information is included in the dose-response modeling, so the modeling takes the entire set of tumor incidence data into account.

Thus, the NTP (1995) female mouse thyroid tumor data supports the conclusion that TBAc exposure poses a human cancer risk at environmentally-relevant concentrations.

Summary

The information described above, in combination with the mixed TBA genotoxicity data, indicates that TBA should not be assumed to be a hormonally-mediated thyroid carcinogen with a threshold for effect in mice, and the female mouse thyroid follicular cell tumor data reported by NTP (1995) is relevant for use in determining the potential human cancer risk from TBA exposure.

VI. QUANTITATIVE CANCER RISK ASSESSMENT

Prior OEHHA TBA and TBAc Cancer Risk Assessments

OEHHA previously calculated an animal CSF (CSF_{animal}) for TBA from the Cirvello (1995) TBA male rat kidney tumor data using TOX_RISK version 3.5 software (OEHHA, 1999a). In that evaluation, the CSF_{animal} cancer potency was derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}), where CSF_{animal} = 10 percent/LED_{10}. The potency estimate was converted to human equivalents [CSF_{human} in $(mg/kg\text{-}day)^{-1}$] using body weight scaling $(BW)^{3/4}$. The TBA CSF_{human} value, 3×10^{-3} $(mg/kg\text{-}day)^{-1}$, was calculated using data from the most sensitive species and sex; in this case from the NTP (1995) male F344 rat kidney tumor data.

A CSF_{human} of 2×10^{-3} $(mg/kg\text{-}day)^{-1}$ was then derived for TBAc, assuming 100% metabolism of TBAc to the presumptive human metabolite, TBA, and a MWR of 0.64 (TBA molecular weight 74.12 / TBAc molecular weight 116.16). An IUR for TBAc of 4×10^{-7} $(\mu g/m^3)^{-1}$ was derived from the CSF_{human} value for TBAc by assuming a human breathing rate (BR) of 20 m^3/day, 70% FA, and an average human body weight of 70 kg. These CSF_{human} and IUR values for TBAc were included in a 2006 report by the California Air Resources Board (ARB, 2006).

Current OEHHA TBAc Cancer Risk Assessment

The final NTP report for TBA (1995) listed exposed dose values that were slightly different from the values published in Cirvello *et al.* (1995) that were used to derive OEHHA's 1999 CSF for TBA. Lifetime average daily dose estimates from the NTP TBA drinking water exposure study (1995) were reported in Cirvello *et al.* (1995) as 0, 85, 195 or 420 mg/kg-day for male rats, and 0, 510, 1015, or 2105 mg/kg-day for female mice, and were reported in NTP (1995) as 0, 90, 200, or 420 mg/kg-day for male rats, and 0, 510, 1020, or 2110 mg/kg-day for female mice.

The administered and exposed doses and tumor incidence data uncorrected for mortality for both tumor types are listed in Table 10, and are from NTP (1995). Table 17 lists the administered and exposed doses and the lifetime tumor incidence adjusted for effective number of animals for male rat kidney and female mouse thyroid follicular cell tumors from the 1995 NTP TBA study. The effective number of animals is the number of animals alive on the first day the tumor of interest was observed in any dose group. Effective number of animals is generally suitable for performing cancer dose-response analyses, unless an additional tumor incidence correction (e.g. poly-3 correction) is required for substantial early mortality before the first tumor of interest is observed. No additional corrections for the potential impact of early mortality on tumor incidence were required, as a survival analysis performed by NTP (1995; Tables 9 and 11 herein) indicated that there was no significant survival difference between the control groups and the dose groups showing significantly increased tumor incidences.

Table 17. Increased tumor incidences adjusted for effective number of animals in Fischer 344 male rats and female B6C3F$_1$ mice exposed to *tertiary*-butanol in drinking water (NTP, 1995).

Sex, species	Tumor type	Administered dose	Exposed dose (mg/kg-day)	Tumor incidencea	% Tumor incidence	First tumor incidence (days)
Male rats	Renal tubule adenomas/ carcinomas	0	0	8/47	17	553
		1.25	90	13/45	28.9	624
		2.5	200	19/43**	44.2	525
		5	420	13/46	28.3	566
Female mice	Thyroid follicular cell adenomas	0	0	2/47+	4.3	729 (T)
		5	510	3/47	6.4	729 (T)
		10	1020	2/51	3.9	729 (T)
		20	2110	9/53*	17	646

[a]numerator = number of tumors, denominator = effective number of animals
(T): terminal sacrifice
Fisher exact test pairwise comparison with controls: * $p = 0.047$; ** $p = 0.004$
Cochran-Armitage trend test for dose response: + $p = 0.007$

The tumor incidence data was the same for both Cirvello (1995) and NTP (1995). TBA CSFs can be calculated from the NTP (1995) TBA drinking water exposure study male rat kidney and female mouse thyroid follicular cell tumor data sets using the Multistage Cancer Model function of BMDS version 2.6 (U.S. EPA, 2017). A CSF_{animal} could not be derived from the NTP (1995) male rat kidney tumor data set with the high dose group included in the analysis due to lack of model convergence. Therefore, a CSF_{animal} of 3.1×10^{-3} $(mg/kg\text{-}day)^{-1}$ was calculated from the NTP male rat kidney tumor data set with the high dose (420 mg/kg-day) removed, using a 1st degree polynomial (a goodness-of-fit *p* value could not be determined for a 2nd degree polynomial). Removing the male rat kidney tumor high dose group from the dose-response analysis was done based on modeling rather than biological considerations. US EPA (2012) has stated that dropping a dose group (usually the highest dose group) from a tumor incidence data set is acceptable when necessary to obtain an adequate model fit.

The NTP (1995) female mouse thyroid tumor data set was evaluated using both 2nd degree and 3rd degree polynomial multistage cancer models. The 3rd degree polynomial multistage cancer model provided a better fit of that dataset (based on lower Akaike Information Criterion (AIC) values and a higher *p*-value), and was used to calculate a CSF_{animal} of 8×10^{-5} $(mg/kg\text{-}day)^{-1}$ from the female mouse thyroid tumor data set. All CSF_{animal} values (for both rats and mice) were derived using a Benchmark Response Value of 5% (5% extra risk) to calculate the Benchmark Dose (BMD; OEHHA, 2008, 2009).

A summary of the BMDS output is listed in Table 18. The associated graphic representation of the model results for the NTP (1995) male F344 rat renal tubule adenomas and carcinomas data set is provided in Figure 8.

Table 18. BMDS output from an analysis of the NTP (1995) *tertiary*-butanol tumor data.

Sex/strain/species/tumor site	BMD mg/kg-day	BMDL mg/kg-day	Goodness of fit *p*-value	AIC
Male F344 rat renal tubule adenomas/carcinomas; high dose dropped	26.51	16.36	0.84	160.06
Female $B6C3F_1$ mouse thyroid follicular cell adenomas	1531.38	625.54	0.71	108.73

AIC: Akaike Information Criterion
BMD: Benchmark Dose
BMDL: lower 95% confidence limit on the BMD

The animal TBA CSF value of 3.1×10^{-3} $(mg/kg\text{-}day)^{-1}$ was then determined using the TBA CSF from the most sensitive species and sex, in this case, from the NTP (1995) male F344 rat kidney tumor data. The potency estimate for TBA was converted to human equivalents [in $(mg/kg\text{-}day)^{-1}$] using body weight $(BW)^{3/4}$ scaling. A time-weighted average body weight for the control rats (0.431 kg) was calculated from information presented by NTP (1995) for control animals during the study, and a default human body weight of 70 kg was used. The resulting CSF_{human} value of 1.1×10^{-2} $(mg/kg\text{-}day)^{-1}$ was determined for TBA, using the calculation below.

$$TBA\ CSF_{human} = TBA\ CSF_{animal} \times (BW_{human} \div BW_{animal})^{1/4}$$

$$= 3.1 \times 10^{-3}\ (mg/kg\text{-}day)^{-1} \times (70\ kg \div 0.431\ kg)^{1/4}$$

$$= 1.1 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}$$

The absorption and metabolism to TBA for a 6-hr dose at 100 ppm of TBAc was derived from the rodent data presented in Cruzan and Kirkpatrick (2006). These data indicate that 50.7 mg/kg of the radiolabeled TBAc was inhaled over 6 hours, and within 7 days after exposure ended, 92.6% of the radioactivity was recovered in urine, feces and tissues, and 4.8% of the radioactivity was recovered in exhaled air. Within the first 12 hours after exposure, 4.5% of the radioactivity (95% of the total amount exhaled) was exhaled suggesting that this minor route of excretion could occur before or after absorption through lung tissue. Therefore, it is reasonable to assume that at least 95% of TBAc inhaled over 6 hours is absorbed to blood, and indicating that 0.95 would be an appropriate FA value for inhaled TBAc.

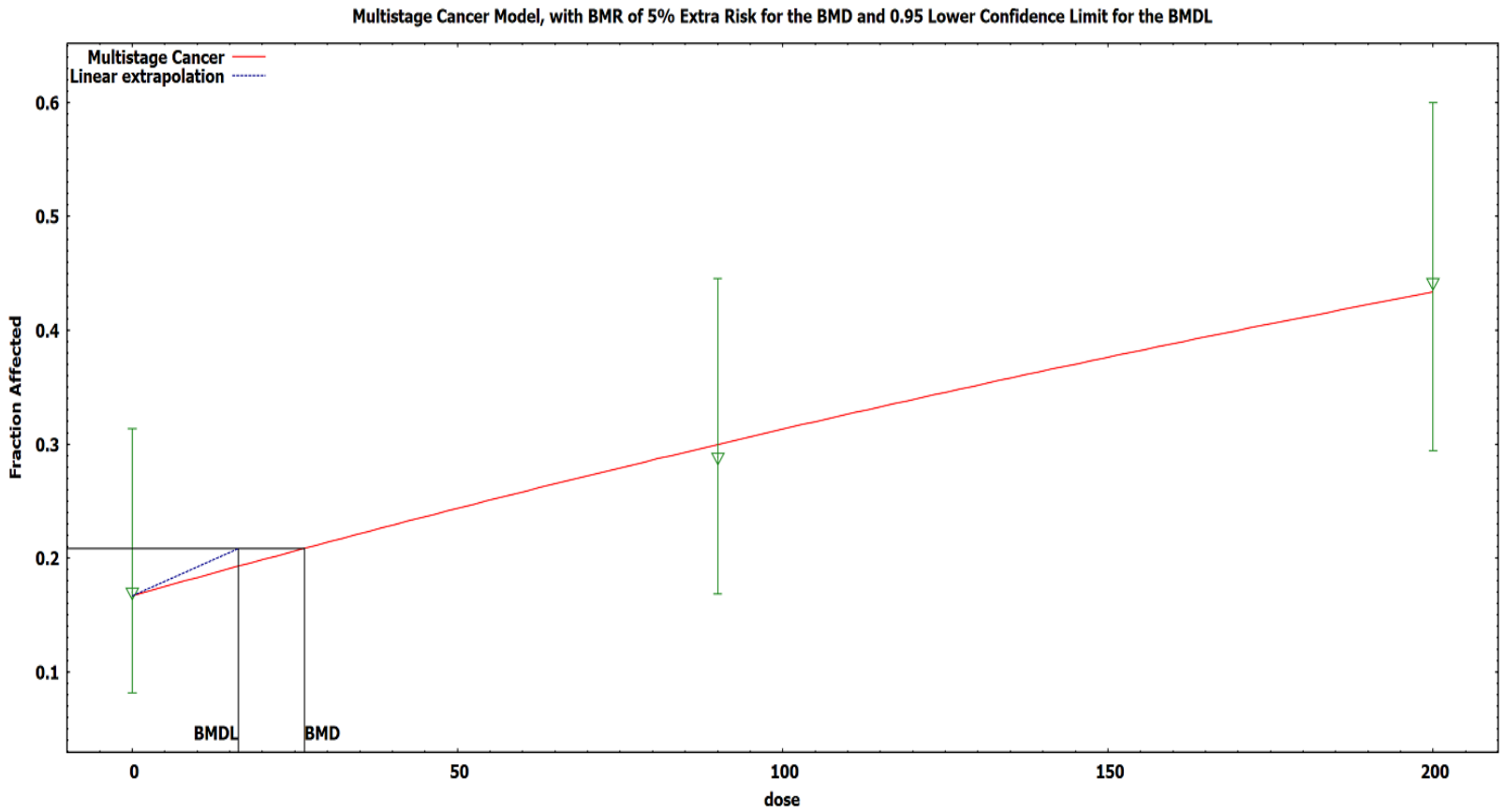

Figure 8. BMDS Multistage Cancer Model Data Plot for NTP (1995) Male Rat Kidney Adenomas/Carcinomas.

Additionally, Table V and Figure 1 in the Cruzan and Kirkpatrick (2006) report shows that 3.5% plus 1.3% of TBAc that is excreted through the urinary pathway converts to *t*-butyl-2-hydroxyacetate or unidentified metabolites, and 24.2% converts to 2-hydroxymethyl-isopropyl acetate. This leaves 71% of urinary products of metabolism that could originate from TBA, indicating that 0.71 would be an appropriate metabolic conversion factor (TBAc MC) for conversion of TBA to TBAc. In the absence of fecal and tissue TBAc quantitative metabolite data, it can be reasonably assumed that the percentage of metabolism products that could originate from TBA in those compartments is similar to the percentage found in the urinary compartment. It should be noted that the TBA cancer bioassay was performed using F344 rats, while the TBA metabolism studies were done in Sprague-Dawley rats. Possible differences in TBA metabolism between the rat strains are a potential source of uncertainty in the TBAc oral CSF calculation presented below.

An oral TBAc CSF of 5.0×10^{-3} $(mg/kg\text{-}day)^{-1}$ was derived from the TBA CSF_{human} value of 1.1×10^{-2} $(mg/kg\text{-}day)^{-1}$, assuming a TBAc MC of 0.71 (derived above) for conversion of TBAc to TBA and a MWR of 0.64 (TBA molecular weight 74.12 / TBAc molecular weight 116.16), using the following relationship:

$$TBAc\ CSF_{oral} = TBA\ CSF_{human} \times TBAc\ MC \times MWR$$

$$= 1.1 \times 10^{-2}\ (mg/kg\text{-}day)^{-1} \times 0.71 \times 0.64$$

$$= 5.0 \times 10^{-3}\ (mg/kg\text{-}day)^{-1}$$

An inhalation TBAc CSF of 4.7×10^{-3} $(mg/kg\text{-}day)^{-1}$ was calculated from the oral TBAc CSF using the following relationship, where FA = 95% fractional absorption (derived as described above):

$$TBAc\ CSF_{inhalation} = TBAc\ CSF_{oral} \times FA$$

$$= 5.0 \times 10^{-3}\ (mg/kg\text{-}day)^{-1} \times 0.95$$

$$= 4.7 \times 10^{-3}\ (mg/kg\text{-}day)^{-1}$$

A TBAc IUR of 1.3×10^{-6} $(\mu g/m^3)^{-1}$ was then derived from the inhalation CSF value for TBAc by assuming a human BR of 20 m^3/day, an average human body weight (BW) of 70 kg, and a mg-to-µg conversion (CV) of 1000 using the following relationship:

$$\text{TBAc IUR} = \frac{CSF_{inhalation} \times BR}{BW \times CV}$$

$$= \frac{4.7 \times 10^{-3}\ \text{kg-day}}{\text{mg}} \times \frac{20\ \text{m}^3}{\text{day}} \times \frac{1}{70\ \text{kg}} \times \frac{1\ \text{mg}}{1000\ \mu\text{g}}$$

$$= 1.3 \times 10^{-6}\ \text{m}^3/\mu\text{g}$$

$$= 1.3 \times 10^{-6}\ (\mu\text{g/m}^3)^{-1}$$

The TBA and TBAc CSFs used to calculate the TBAc IUR are summarized in Table 19, below.

Table 19. *Tertiary*-butanol (TBA) and *tertiary*-butyl acetate (TBAc) cancer slope factors (CSFs) used to calculate the TBAc inhalation unit risk factor (IUR).

TBA and TBAc CSFs and IUR	
TBA CSF_{animal} $(mg/kg\text{-}day)^{-1}$	3.1×10^{-3}
TBA CSF_{human} $(mg/kg\text{-}day)^{-1}$	1.1×10^{-2}
TBAc CSF_{oral} $(mg/kg\text{-}day)^{-1}$	5.0×10^{-3}
TBAc $CSF_{inhalation}$ $(mg/kg\text{-}day)^{-1}$	4.7×10^{-3}
TBAc IUR $(\mu g/m^3)^{-1}$	1.3×10^{-6}

To relate the TBAc IUR to potential cancer risks in California, OEHHA estimated cancer risk based on potential environmental concentrations of TBAc. Our results indicate that at a theoretical TBAc air concentration of 1 $\mu g/m^3$ (0.21 ppb), cancer risk is 1.3 in 1 million. ARB (2006) estimated potential outdoor air concentrations of TBAc in Southern California resulting from unrestricted use of TBAc to be 2.8 $\mu g/m^3$ (0.59 ppb). This estimate used data from the most populated and polluted air basin in California (South Coast Air Basin) with the most emission and air quality data at the time of their report. Lacking measurements of TBAc, ARB used emissions from surrogate compounds (toluene, methyl ethyl ketone, and m-, o-, and p- xylenes) in their models because those emissions represent over half of the mass of US EPA regulated VOCs that could be replaced by TBAc. Differences in atmospheric chemistry and lifetime were not taken into account. In this scenario, using the TBAc IUR to calculate cancer risk, the potential increase in cancer risk resulting from the use of TBAc in Southern California is 3.6 in 1 million.

VII. CONCLUSIONS

No carcinogenicity data are available for TBAc. However, TBAc has been demonstrated to be substantially metabolized to TBA in rats (Groth and Freundt, 1994). TBA genotoxicity data are mixed, but positive DNA damage (Tang *et al.*, 1997; Sgambato *et al.*, 2009) and bacterial gene mutation studies for TBA (Williams-Hill *et al.*, 1999) suggest that TBA may cause oxidative DNA damage resulting in the

induction of gene mutations. TBA has been shown to induce tumors in both rats and mice (NTP, 1995; Cirvello *et al*., 1995), and OEHHA (1999a) has previously calculated a human CSF for TBA, a presumptive human metabolite, of 3×10^{-3} $(mg/kg\text{-}day)^{-1}$, corresponding to an IUR of 9×10^{-7} $(\mu g/m^3)^{-1}$. (This was part of an expedited evaluation supporting a drinking water Action Level for TBA developed by OEHHA (1999a) and adopted by the Department of Health Services.) This raises a concern that exposure to TBAc may result in a cancer risk because of its presumed metabolic conversion to TBA in humans.

It has been proposed that the NTP (1995) TBA carcinogenicity data may not be relevant to human cancer risk assessment because 1) the male rat kidney tumors are the result of TBA-induced α2u nephropathy, a pathological effect specific for male rats, and 2) the female mouse thyroid follicular cell tumors may be due to an effect on thyroid hormone levels, which would only occur above a threshold higher than the level of expected human exposures. However, the data pertaining to both these possibilities are insufficient to allow the determination that the NTP (1995) TBA carcinogenicity data are not relevant to human cancer risk assessment. Therefore, TBAc should be considered to pose a potential cancer risk to humans.

In this document, OEHHA calculated TBA CSFs from the NTP (1995) TBA drinking water exposure study's male rat kidney and female mouse thyroid follicular cell tumor data sets using BMDS version 2.6 (U.S. EPA, 2015). CSF_{animal} values of 3.1×10^{-3} $(mg/kg\text{-}day)^{-1}$ and 8.0×10^{-5} $(mg/kg\text{-}day)^{-1}$ were derived from the male F344 rat kidney tumor data and the female mouse thyroid tumor data, respectively. The animal TBA CSF value of 3.1×10^{-3} $(mg/kg\text{-}day)^{-1}$ was then determined using the TBA CSF from the most sensitive species and sex; in this case, from the NTP (1995) male F344 rat kidney tumor data. This potency estimate was converted to human equivalents [in $(mg/kg\text{-}day)^{-1}$] using body weight $(BW)^{3/4}$ scaling. The resulting TBA CSF_{human} value was 1.1×10^{-2} $(mg/kg\text{-}day)^{-1}$.

A human oral CSF of 5.0×10^{-3} $(mg/kg\text{-}day)^{-1}$ was calculated for TBAc, assuming 71% metabolism of TBAc to TBA and a MWR of 0.64 (TBA molecular weight 74.12 / TBAc molecular weight 116.16). An inhalation TBAc CSF of 4.7×10^{-3} $(mg/kg\text{-}day)^{-1}$ was calculated from the oral TBAc CSF assuming 95% FA. A TBAc IUR of 1.3×10^{-6} $(\mu g/m^3)^{-1}$ was then derived from the TBAc inhalation CSF value by assuming a human BR of 20 m^3/day, and an average human body weight of 70 kg.

The factors causing the change from the prior TBAc IUR (ARB, 2006) to the updated TBAc IUR in this document are:

1. The TBA CSF used to calculate the prior TBAc IUR was derived using the linearized multistage procedure contained in TOX_RISK version 3.5 (ARB, 2006), using all dose groups from the NTP (1995) male rat kidney tumor incidence data set. The current TBA CSF was derived using US EPA BMDS version 2.6, the high dose group (420 mg/kg-day) was dropped from the NTP male rat kidney tumor data set, and the model was fit to the data using a 1st degree polynomial.

2. The current TBAc IUR calculations apply an effective number of animals adjustment to the NTP (1995) male rat kidney tumor incidence data set.

3. The prior TBAc IUR calculations assumed that 70% of inhaled TBAc is absorbed, and 100% of absorbed TBAc is metabolized to TBA. The current TBAc IUR calculations assume that 95% of inhaled TBAc is absorbed, and 71% of absorbed TBAc is metabolized to the presumptive human metabolite, TBA.

VIII. REFERENCES

Aarstad K, Zahlsen K and Nilsen OG. 1985. Inhalation of butanols: changes in the cytochrome P450 enzyme system. In: Receptors and Other Targets for Toxic Substances. Proceedings of the European Society of Toxicology Meeting held in Budapest, June 11-14, 1984. Arch Toxicol Suppl 8:418-421.

Air Resources Board (ARB). 2006. Environmental Impact Assessment of *Tertiary*-Butyl Acetate. Air Resources Board, Sacramento, CA.

Air Resources Board (ARB). 2010. 2010 California Toxics Inventory Summary. http://www.arb.ca.gov/toxics/cti/cti.htm.

Air Resources Board (ARB). 2017. AB 2588 Air Toxics "Hot Spots" Program 2015 Facility Emissions and Risk Data. https://www.arb.ca.gov/app/emsinv/facinfo/facinfo.php

Amberg A, Rosner E and Dekant W. 1999. Biotransformation and kinetics of excretion of methyl-*tert*-butyl ether in rats and humans. Toxicol Sci 51:1-8.

Amberg A, Rosner E and Dekant W. 2000. Biotransformation and kinetics of excretion of ethyl *tert*-butyl ether in rats and humans. Toxicol Sci 53:194-201.

Amberg A, Rosner E and Dekant W. 2001. Toxicokinetics of methyl-*tert*-butyl ether and its metabolites in humans after oral exposure. Toxicol Sci 61: 62-67.

Bechtel DH and Cornish HH. 1972. Effect of the butyl alcohols on liver microsomal enzymes. In: Abstracts of papers for the Eleventh Annual Meeting of the Society of Toxicology, Williamsburg VA, March 5-9, 1972. Toxicol Appl Pharmacol 22:298-299.

Bernauer U, Amberg A, Scheutzow D and Dekant W. 1998. Biotransformation of ^{12}C- and 2-^{13}C-labeled methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-butyl alcohol in rats: Identification of metabolites in urine by ^{13}C Nuclear Magnetic Resonance and Gas Chromatography/Mass Spectrometry. Chem Res Toxicol 11: 651-658.

Blanck O, Fowles J, Schorsch F, Pallen C, Espinasse-Lormeau H, Schulte-Koerne E, Totis M and Banton M. 2010. *Tertiary* butyl alcohol in drinking water induces phase I and II liver enzymes with consequent effects on thyroid hormone homeostasis in the B6C3F1 female mouse. J Appl Toxicol 30:125-132.

Borghoff SJ and Asgharian B. 1996. Ethyl tertiary-Butyl Ether (ETBE): Pharmacokinetic Study in Male and Female CD-1 Mice After Single Inhalation Exposure and Male and Female F-344 Rats After Single and Repeated Inhalation Exposure. Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC.

Borghoff SJ, Prescott JS, Janszen DB, Wong BA and Everitt JI. 2001. α2u-Globulin nephropathy, renal cell proliferation, and dosimetry of inhaled *tert*-butyl alcohol in male and female F-344 rats. Toxicol Sci 61:176-186.

Bus JS, Banton MI, Faber WD, Kirman CR, McGregor DB and Pourreau DB. 2015. Human health screening level risk assessments of *tertiary*-butyl acetate (TBAc): calculated acute and chronic reference concentration (RfC) and Hazard Quotient (HQ) values based on toxicity and exposure scenario evaluations. Crit Rev Toxicol 45: 142-71.

Cain WS and Schmidt R. 2009. Can we trust odor databases? Example of t- and n-butyl acetate. Atmos Environ 43: 2591-2601.

Cirvello JD, Radovsky A, Heath JE, Farnell DR and Lindamood C 3rd. 1995. Toxicity and carcinogenicity of *t*-butyl alcohol in rats and mice following chronic exposure in drinking water [published erratum appears in Toxicol Ind Health 1995 May-Jun;11(3):363]. Toxicol Ind Health 11:151-165.

Cox DR. 1972. Regression models and life tables. J R Stat Soc Series B Stat Methodol 34:187-220.

Cruzan G and Kirkpatrick D. 2006. *Tertiary*-butyl acetate metabolism, toxicity, and human health considerations. Toxicol Env Chem 88:405-421.

Curran PG and DeGroot LJ. 1991. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev 12:135-150.

Doi AM, Hill G, Seely J, Hailey JR, Kissling G and Bucher JR. 2007. α2u-globulin nephropathy and renal tumors in National Toxicology Program studies. Tox Path 35:533–540.

Essig KM, Groth G and Freundt KJ. 1989. Different elimination of n-butyl acetate and *t*-butyl acetate. Arch Pharmacol 340:R33.

European Chemicals Agency (ECHA). 2017a. *Tert*-butyl acetate: EC number: 208-760-7 | CAS number: 540-88-5. Acute Toxicity: dermal. Retrieved Sept. 08, 2017, from https://echa.europa.eu/registration-dossier/-/registered-dossier/10192/7/3/4.

European Chemicals Agency (ECHA). 2017b. *Tert*-butyl acetate: EC number: 208-760-7 | CAS number: 540-88-5. Skin sensitisation. Retrieved Sep 08, 2017, from https://echa.europa.eu/registration-dossier/-/registered-dossier/10192/7/5/2.

Eustis SL, Hailey JR, Boorman GA, and Haseman JK. 1994. The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. Toxicol Pathol 22:457-472.

Faber W, Kirkpatrick D, Coder P, Li A, Borghoff S and Banton M. 2014. Subchronic, reproductive, and maternal toxicity studies with *tertiary* butyl acetate (TBAc). Reg Toxicol Pharmacol 68:332-342.

Groth G and Freundt KJ. 1994. Inhaled *tert*-butyl acetate and its metabolite *tert*-butyl alcohol accumulate in the blood during exposure. Hum Exp Toxicol 13:478-480.

Hard GC, Bruner RH, Cohen SM, Pletcher JM, and Regan KS. 2011. Renal histopathology in toxicity and carcinogenicity studies with *tert*-butyl alcohol administered in drinking water to F344 rats: A pathology working group review and re-evaluation. Regul Toxicol Pharmacol 59:430-436.

Haseman JK and Lockhart A. 1994. The relationship between use of the Maximum Tolerated Dose and study sensitivity for detecting rodent carcinogenicity. Fund Appl Tox 22:382-391.

Hazardous Substances Data Bank (HSDB; internet version). 2017. *Tert*-butyl acetate, CASRN: 540-88-5. Retrieved Aug. 09, 2017, from https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~a4mFvi:1.

Hong JY, Yang CS, Lee M, Wang YY, Huang WQ, Tan Y, Patten CJ and Bondoc FY. 1997. Role of cytochromes P450 in the metabolism of methyl *tert*-butyl ether in human livers. Arch Toxicol 71:266-9.

Huntingdon Life Sciences Limited. 2000a. *Tertiary* Butyl Acetate Bacterial Mutation Assay. Prepared by Huntingdon Life Sciences Ltd. for Lyondell Chemicals Worldwide, Newtown Square, PA.

Huntingdon Life Sciences Limited. 2000b. ^{14}C-[*Tertiary*-Butyl-Acetate] Metabolism and Pharmacokinetic Study in the Rat After Inhalation. Prepared by Huntingdon Life Sciences Ltd. for Lyondell Chemicals Worldwide, Newtown Square, PA.

Industrial Biotest Laboratories Inc. 1958. Toxicity Studies on TFA-168. *Tert*-Butyl Acetate. Performed by Industrial Bio-Test Laboratories, Inc., Northbrook IL for Albemarle Corporation, Baton Rouge LA.

International Agency for Research on Cancer (IARC). 1999. Consensus Report. In: Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publications No. 147. Capen CC, Dybing E, Rice JM and Wilbourn JD, eds. IARC, Lyon, France, pp.1-14.

Johnson EM, Christian MS, Dansky L and Gabel BEG. 1987. Use of the adult developmental relationship in prescreening for developmental hazards. Teratog, Carcinog, and Mutagen. 7: 273-285.

Jones RD, Taylor AM, Tong EY and Repa JJ. 2013. Carboxylesterases are uniquely expressed among tissues and regulated by nuclear hormone receptors in the mouse. Drug Metab Dispos 41:40-9.

Lindamood C, Farnell DR, Giles HD, Prejean JD, Collins JJ, Takahashi K and Maronpot RR. 1992. Subchronic toxicity studies of *t*-butyl alcohol in rats and mice. Fundam Appl Toxicol 19:91-100.

Lyondell Chemical Co. 2001. Supplemental Information on *Tertiary* Butyl Acetate. Submitted to the California Air Resources Board. Lyondell Chemical Co, Houston, TX.

MAK Commission. 2016. The MAK Collection for Occupational Health and Safety: *tert*-Butyl acetate. Wiley Online Library. 1:578-596.

Maronpot RR, Yoshizawa K, Nyska A, Harada T, Flake G, Mueller G, Singh B, Ward JM. 2010. Hepatic enzyme induction: histopathology. Toxicol Pathol 38:776-795.

McClain RM. 2001. Assessment of the Thyroid Follicular Cell Tumor Findings from Toxicity and Carcinogenicity Studies with *tert*-Butyl Alcohol in B6C3F1 Mice. Prepared for Lyondell Chemical Co, Houston, TX.

McGregor DB, Cruzan G, Callander RD and Banton M. 2005. The mutagenicity of *tertiary*-butyl acetateTM and methyl *tertiary*-butyl ether in *Salmonella typhimurium.* Mutat Res 565:181-189.

McGregor D and Hard GC. 2001. Renal tubule tumor induction by *tertiary*-butyl alcohol. Toxicol Sci 61:1-3.

Melnick RL, Burns KM, Ward JM and Huff J. 2012. Chemically exacerbated chronic progressive nephropathy not associated with renal tubular tumor induction in rats: An evaluation based on 60 carcinogenicity studies by the Nation Toxicology Program. Toxicol Sci 128:346-356.

Melnick RL, White MC, Davis JM, Hartle RW, Ghanayem B, Ashley DL, Harry GJ, Zeiger E, Shelby M and Ris CH. 1997. Potential Health Effects of Oxygenated Gasoline. In: Interagency Assessment of Oxygenated Fuels. Coordinated by the Office of Science and Technology Policy, Washington, DC.

Nagata Y. 2003. Measurement of Odor Threshold by Triangle Odor Bag Method. Government of Japan.

National Toxicology Program (NTP). 1995. Toxicology and Carcinogenesis Studies of *t*-Butyl Alcohol (CAS No. 75-65-0) In F344/N Rats and B6C3F$_1$ Mice (Drinking Water Studies). NTP TR436, NIH Publication No. 95-3167. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP). 1997. Toxicity Studies of *t*-Butyl Alcohol (CAS No. 75-65-0) Administered by Inhalation to F344/N Rats and B6C3F1 Mice. NTP TR053, NIH Publication 97-3942. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

National Toxicology Program (NTP). 2017a. Historical Controls. National Institute of Environmental Health Sciences, Research Triangle Park, NC. (http://ntp.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/r_orlwr.txt

National Toxicology Program (NTP). 2017b. Organ Sites with Neoplasia database. National Institute of Environmental Health Sciences, Research Triangle Park, NC. https://manticore.niehs.nih.gov/organsites

Nihlén A, Lof A and Johanson G. 1995. Liquid/air partition coefficients of methyl and ethyl *t*-butyl ethers, *t*-amyl methyl ether, and *t*-butyl alcohol. J Expo Anal Environ Epidemiol 5:573-82.

Nihlén A, Lof A and Johanson G. 1998. Controlled ethyl *tert*-butyl ether (ETBE) exposure of male volunteers. I. Toxicokinetics. Toxicol Sci 46:1-10.

Nihlén A, Sumner SC, Lof A and Johanson G. 1999. $^{13}C(2)$-Labeled methyl *tert*-butyl ether: Toxicokinetics and characterization of urinary metabolites in humans. Chem Res Toxicol 12:822-30.

Office of Environmental Health Hazard Assessment (OEHHA). 1999a. Expedited evaluation of risk assessment for *tertiary* butyl alcohol in drinking water. Memorandum from GV Alexeeff (Office of Environmental Health Hazard Assessment, California Environmental Protection Agency) to DP Spath (California Department of Health Services) (memorandum dated 6/2/99).

Office of Environmental Health Hazard Assessment (OEHHA). 1999b. Health effects of exposure to methyl *tertiary* butyl ether (MTBE). Air Toxicology and Epidemiology Section, Oakland, CA.

Office of Environmental Health Hazard Assessment (OEHHA). 2000. Potential human health effects associated with *tertiary*-butyl acetate. Memorandum from M Marty, OEHHA to J Brooks, Air Resources Board. Air Toxicology and Epidemiology Section, Oakland, CA.

Office of Environmental Health Hazard Assessment (OEHHA). 2009. Air Toxics Hot Spots Risk Assessment Guidelines Technical Support Document for Cancer Potency Factors. Air Toxicology and Epidemiology Branch, Oakland, CA.

Prescott-Mathews JS, Wolf DC, Wong BA and Borghoff SJ. 1997. Methyl *tert*-butyl ether causes α2u-globulin nephropathy and enhanced renal cell proliferation in male Fischer-344 rats. Toxicol Appl Pharmacol 143:301-314.

Redinbo MR and Potter PM. 2005. Mammalian carboxylesterases: from drug targets to protein therapeutics. Drug Discov Today 10:313-25.

Saito A, Sasaki T, Kasai T, Katagiri T, Nishizawa T, Noguchi T, Aiso S, Nagano K and Fukushima S. 2013. Hepatotumorigenicity of ethyl *tertiary*-butyl ether with 2-year inhalation exposure in F344 rats. Arch Toxicol 87:905-914.

Sgambato A, Iavicoli I, De Paola B, Bianchino G, Boninsegna A, Bergamaschi A, Pietroiusti A and 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.

Sontag JR, Page NP and Safiotti U. 1976. Guidelines for Carcinogen Bioassay in Small Rodents. DHHS Publication (NIH) 76-801. National Cancer Institute, Bethesda, MD.

Stillmeadow, Inc. 1997. Acute Inhalation Toxicity Study in Rats. Study # 3639-97; Project # MB 97-6119.05. Stillmeadow, Inc. Sugar Land, TX

Swenberg JA. 1993. Alpha 2u-globulin nephropathy: review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Environ Health Perspect 101: 39-44.

Swenberg JA and Lehman-McKeeman LD. 1999. α2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. In: Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publications No. 147. Capen CC, Dybing E, Rice JM and Wilbourn JD, eds. IARC, Lyon, France, pp. 95-118.

Takahashi K, Lindamood C, 3rd and Maronpot RR. 1993. Retrospective study of possible alpha-2 mu-globulin nephropathy and associated cell proliferation in male Fischer 344 rats dosed with *t*-butyl alcohol. Environ Health Perspect 101 Suppl 5:281-285.

Tang G, Wang J and Zhuang Z. 1997. [Cytotoxicity and genotoxicity of methyl *tert*-butyl ether and its metabolite to human leukemia cells]. Zhonghua yu fang yi xue za zhi [Chinese Journal of Preventive Medicine] 31:334-337.

Tarone RE. 1975. Tests for trend in life table analysis. Biometrika 62:679-682.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC and Sasaki YF. 2000. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen 35:206-221.

US EPA. 2012. Benchmark Dose Technical Guidance. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460.

US EPA. 2017. Benchmark Dose Software (BMDS) version 2.6. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Research Triangle Park, NC 27711.

Williams-Hill D, Spears CP, Prakash S, Olah GA, Shamma T, Moin T, Kim LY and Hill CK. 1999. Mutagenicity studies of methyl-*tert*-butyl ether using the Ames tester strain TA102. Mutat Res 446:15-21.

Williams TM and Borghoff SJ. 2001. Characterization of *tert*-butyl alcohol binding to α2u-globulin in F-344 rats. Toxicol Sci 62:228-235.

Yang YS, Ahn TH, Lee JC, Moon CJ, Kim SH, Park SC, Chung YH, Kim HY and Kim JC. 2007. Effects of *tert*-butyl acetate on maternal toxicity and embryo-fetal development in Sprague-Dawley rats. Birth Defects Res B Dev Reprod Toxicol 80: 374-82.

Yang YS, Lee J, Kwon S, Seo HS, Choi SJ, Yu HJ, Song JA, Lee K, Lee BS, Heo JD, Cho KH and Song CW. 2010. LC_{50} determination of *tert*-butyl acetate using a nose only inhalation exposure in rats. Toxicol Res 26: 293-300.

Yuan Y, Wang HF, Sun HF, Du HF, Xu LH, Liu YF, Ding XF, Fu DP and Liu KX. 2007. Adduction of DNA with MTBE and TBA in mice studied by accelerator mass spectrometry. Environ Toxicol 22:630-635.