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Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)

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EXECUTIVE SUMMARY

Summation of Occurrence and Health Effects

tert-Butanol is produced by humans for multiple purposes, such as a solvent for paints, a denaturant for ethanol and several other alcohols, an agent for dehydrating, and in the manufacture of flotation agents, fruit essences, and perfumes. *tert*-Butanol also is a primary metabolite of methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE). Exposure to *tert*-butanol occurs primarily through breathing air containing *tert*-butanol vapors and consuming contaminated water or foods. Exposure can also occur through direct skin contact.

Animal studies demonstrate that chronic oral exposure to *tert*-butanol is associated with kidney and thyroid effects. No chronic inhalation exposure studies have been conducted. Evidence is suggestive of carcinogenic potential for *tert*-butanol, based on thyroid tumors in male and female mice and renal tumors in male rats.

EFFECTS OTHER THAN CANCER OBSERVED FOLLOWING ORAL EXPOSURE

Kidney effects are a potential human hazard of oral exposure to *tert*-butanol. Kidney toxicity was observed in males and females in two strains of rats. Kidney weights were increased in male and female rats after 13 weeks or 15 months of treatment. Histopathological examination in male and female rats showed increased incidence or severity of nephropathy after 13 weeks of oral exposure, increases in severity of nephropathy after 2 years of oral exposure, and increased transitional epithelial hyperplasia after 2 years of oral exposure. Additionally, increased suppurative inflammation was noted in females after 2 years of oral exposure. In one strain of mice, the only kidney effect observed was an increase in kidney weight (absolute or relative) in female mice after 13 weeks, but no treatment-related histopathological lesions were reported in the kidneys of male or female mice at 13 weeks or 2 years. A mode of action (MOA) analysis determined that *tert*-butanol exposure induces a male rat-specific alpha 2u-globulin-associated nephropathy. *tert*-Butanol, however, is a weak inducer of alpha 2u-globulin nephropathy, which is not the sole process contributing to renal tubule nephropathy. Chronic progressive nephropathy (CPN) might also be involved in some noncancer effects, but the data are complicated by alpha 2u-globulin nephropathy in males. Effects attributable to alpha 2u-globulin nephropathy in males were not considered for kidney hazard identification. Females are not affected by alpha 2u-globulin nephropathy, so changes in kidney weights, transitional epithelial hyperplasia, suppurative inflammation, and severity and incidence of nephropathy in female rats are considered to result from *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

At this time, evidence of selective developmental toxicity, neurodevelopmental toxicity, and reproductive system toxicity following *tert*-butanol exposure is inadequate with minimal effects

observed at otherwise toxic dose levels. The available information also is inadequate to draw conclusions regarding liver and urinary bladder toxicity because of lack of consistency and lack of progression, respectively.

ORAL REFERENCE DOSE (RFD) FOR EFFECTS OTHER THAN CANCER

Kidney toxicity, represented by increases in severity of nephropathy in female rats, was chosen as the basis for the overall RfD (see Table ES-1). The kidney effects observed in female rats in the chronic study by NTP (1995) were used to derive the RfD. Increased severity of nephropathy was selected as the critical effect because it was observed in female rats consistently, it is an indicator of kidney toxicity, and it was induced in a dose-responsive manner. While dose-response modeling was technically feasible, there was uncertainty related to BMR type and values to use for this type of endpoint; accordingly, the point of departure (POD) was derived from the lowest-observed-adverse-effect level (LOAEL) of 43 mg/kg-day (NTP, 1995).

The overall RfD was calculated by dividing the POD for increases in severity of nephropathy by a composite uncertainty factor (UF) of 100 to account for the extrapolation from animals to humans (3), derivation from a LOAEL (3), and for interindividual differences in human susceptibility (10).

Table ES-1. Organ/system-specific oral reference doses (RfDs) and overall RfD for *tert*-butanol

Hazard	Basis	Point of departure[a] (mg/kg-d)	UF	Chronic RfD (mg/kg-d)	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy	43.2	100	4×10^{-1}	Chronic	Medium
Overall RfD	**Kidney**	**43.2**	**100**	**4×10^{-1}**	**Chronic**	**Medium**

[a]Human equivalent dose PODs were calculated using body weight to the ¾ power ($BW^{3/4}$) scaling (U.S. EPA, 2011)

EFFECTS OTHER THAN CANCER OBSERVED FOLLOWING INHALATION EXPOSURE

Kidney effects are a potential human hazard of inhalation exposure to *tert*-butanol. Although no effects were observed in mice, kidney weights were increased in male and female rats following 13 weeks of inhalation exposure. In addition, the severity of nephropathy increased in male rats. No human studies are available to evaluate the effects of inhalation exposure. As discussed above for oral effects, endpoints in males specifically related to alpha 2u-globulin nephropathy were not considered for kidney hazard identification. Changes in kidney weights and

severity of nephropathy in females, however, are considered a result of *tert*-butanol exposure and are appropriate for identifying a hazard to the kidney.

INHALATION REFERENCE CONCENTRATION (RFC) FOR EFFECTS OTHER THAN CANCER

Kidney toxicity, represented by increases in severity of nephropathy, was chosen as the basis for the RfC (see Table ES-2). Although endpoints from a route-specific study were considered, the availability of a physiologically based pharmacokinetic (PBPK) model for *tert*-butanol in rats (Borghoff et al., 2016) allowed for more specific and sensitive equivalent inhalation PODs derived from a route-to-route (RTR) extrapolation from the PODs of the oral NTP (1995) study. The POD adjusted for the human equivalent concentration (HEC) was 491 mg/m^3 based on increases in severity of nephropathy.

As discussed in Section 2.2.2, it is recognized that there is uncertainty in RTR extrapolation because actual risk may not correlate exactly with the internal dose metric used for the extrapolation (in this case, average blood concentration of *tert*-butanol). EPA is not aware of a quantitative analysis of such uncertainty; it would involve comparing cross-route extrapolation to toxicity data for a number of chemicals and endpoints sufficient to characterize the accuracy of the approach. Such an analysis is beyond the scope of this assessment. However, it is EPA's judgment that this uncertainty is less than the uncertainty of the alternative, which would be to base the RfC on the subchronic toxicity data. In particular, toxicity to the kidney requires that *tert*-butanol be systemically distributed in the blood, hence the toxicity must be correlated with some measure of blood concentration. The uncertainty in the extrapolation occurs because the metric used might not accurately predict the effect, versus other possible metrics such as peak concentration.

The RfC was calculated by dividing the POD by a composite UF of 100 to account for toxicodynamic differences between animals and humans (3), derivation from a LOAEL (3), and interindividual differences in human susceptibility (10).

Table ES-2. Organ/system-specific inhalation reference concentrations (RfCs) and overall RfC for *tert*-butanol

Hazard	Basis	Point of departure[a] (mg/m^3)	UF	Chronic RfC (mg/m^3)	Study exposure description	Confidence
Kidney	Increases in severity of nephropathy	491	100	5×10^0	Chronic	Medium
Overall RfC	**Kidney**	**491**	**100**	**5×10^0**	**Chronic**	**Medium**

BMDL = benchmark dose lower confidence limit.

[a]Continuous inhalation HEC that leads to the same average blood concentration of *tert*-butanol as drinking water exposure to the rat at the BMDL.

EVIDENCE OF HUMAN CARCINOGENICITY

Under EPA Cancer Guidelines (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for *tert*-butanol. *tert*-Butanol induced kidney tumors in male (but not female) rats and thyroid tumors (primarily benign) in male and female mice following long-term administration in drinking water (NTP, 1995). The potential for carcinogenicity applies to all routes of human exposure.

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

In accordance with EPA's guidance on alpha 2u-globulin (U.S. EPA, 1991b), rat kidney tumors are unsuitable for quantitative analysis because not enough data are available to determine the relative contribution of alpha 2u-globulin nephropathy and other processes to the overall kidney tumor response. A quantitative estimate of carcinogenic potential from oral exposure to *tert*-butanol was based on the increased incidence of thyroid follicular cell adenomas in female B6C3F1 mice and thyroid follicular cell adenomas and carcinomas in male B6C3F1 mice (NTP, 1995). The study included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting of methods and results.

Although *tert*-butanol was considered to have only *suggestive evidence of carcinogenic potential*, the National Toxicology Program (NTP) study was well conducted and suitable for quantitative analysis. Slope factors were derived for thyroid tumors in female or male mice. The modeled *tert*-butanol POD was scaled to human equivalent doses (HEDs) according to EPA guidance by converting the benchmark dose lower confidence limit corresponding to 10% extra risk ($BMDL_{10}$) on the basis of body weight scaling to the ¾ power ($BW^{3/4}$) (U.S. EPA, 2011, 2005b). Using linear extrapolation from the $BMDL_{10}$, a human equivalent oral slope factor was derived (slope factor = $0.1/BMDL_{10}$). The resulting oral slope factor is **5×10^{-4} per mg/kg-day.**

QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

No chronic inhalation studies of exposure to *tert*-butanol are available. Although the mouse thyroid tumors served as the basis for the oral slope factor, RTR extrapolation is not possible for these thyroid effects in mice because the only PBPK model available is for rats. Therefore, no quantitative estimate of carcinogenic risk could be determined for inhalation exposure.

SUSCEPTIBLE POPULATIONS AND LIFESTAGES FOR CANCER AND NONCANCER OUTCOMES

Information is inadequate to identify any populations or lifestages that might be especially susceptible to *tert*-butanol.

KEY ISSUES ADDRESSED IN ASSESSMENT

This document assesses the human relevance of the kidney effects observed in male and female rats, particularly as the effects relate to alpha 2u-globulin nephropathy and the exacerbation of chronic progressive nephropathy. EPA 1991 and International Agency for Research on Cancer (IARC) 1999 frameworks were used to evaluate whether *tert*-butanol caused alpha 2u-globulin-associated nephropathy (Capen et al., 1999; U.S. EPA, 1991a). The presence of alpha 2u-globulin in the hyaline droplets was confirmed in male rats by alpha 2u-globulin immunohistochemical staining. Linear mineralization and tubular hyperplasia were reported in male rats, although only in the chronic study. Other subsequent steps in the pathological sequence, including necrosis, exfoliation, and granular casts, either were absent or inconsistently observed across subchronic or chronic studies. None of these effects occurred in female rats or in either sex of mice, although these endpoints were less frequently evaluated in these models. Evidence implies that an alpha 2u-globulin MOA is operative, although it is relatively weak in response to *tert*-butanol and is not solely responsible for the renal tubule nephropathy observed in male rats. Chronic progressive nephropathy (CPN) also plays a role in exacerbating nephropathy in both male and female rats. While the etiology of CPN is unknown(NIEHS, 2019; Hard and Khan, 2004; Peter et al., 1986) and it has no known analog in the aging human kidney (NIEHS, 2019; Hard et al., 2009), it cannot be ruled out that a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. chronic kidney disease, diabetic nephropathy, glomerulonephritis, interstitial nephritis, etc.) (NIEHS, 2019). Several other effects in the kidney unrelated to alpha 2u-globulin were observed in female rats, including suppurative inflammation, transitional epithelial hyperplasia, and increased kidney weights (NTP, 1997, 1995). These specific effects observed in female rats, not confounded by alpha 2u-globulin-related processes, are considered the result of *tert*-butanol exposure, and therefore, relevant to humans.

Concerning cancer, alpha 2u-globulin accumulation is indicated as relatively weak in response to *tert*-butanol exposure and not the sole mechanism responsible for the renal tubule carcinogenicity observed in male rats. CPN and other effects induced by both alpha 2u-globulin processes and *tert*-butanol play a role in renal tubule nephropathy, and the evidence indicates that CPN augments the renal tubule tumor induction associated with *tert*-butanol exposure in male rats. Poor dose-response relationships between alpha 2u-globulin processes and renal tumors in male rats and a lack of renal tumors in female rats despite increased CPN severity, however, suggest that other, unknown processes contribute to renal tumor development. Based on this analysis of available MOA data, these renal tumors are considered relevant to humans.

In addition, an increase in the incidence of thyroid follicular cell adenomas was observed in male and female mice in a 2-year drinking water study (NTP, 1995). Thyroid follicular cell hyperplasia was considered a preneoplastic effect associated with the thyroid tumors, and the incidences of follicular cell hyperplasias were elevated in both male and female B6C3F1 mice following exposure. U.S. EPA (1998a) describes the procedures the Agency uses in evaluating

chemicals that are animal thyroid carcinogens. The available evidence base is inadequate for concluding that an antithyroid MOA is operating in mouse thyroid follicular cell tumorigenesis. No other MOAs for thyroid tumors were identified, and the mouse thyroid tumors are considered relevant to humans (U.S. EPA, 1998a).