# 4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

## 4.1 Absorption, distribution, metabolism and excretion

### 4.1.1 *Humans*

No data were available to the Working Group.

### 4.1.2 *Experimental systems*

Two studies of the kinetics and metabolism of 1-*tert*-butoxypropan-2-ol were available. The first was part of the National Toxicology Program (1994) study and involved single oral, intravenous or dermal administrations of [$^{14}$C]1-*tert*-butoxypropan-2-ol to rats and dermal administration of [$^{14}$C]1-*tert*-butoxypropan-2-ol to mice. The second study was conducted in conjunction with the recent National Toxicology Program chronic bioassay (National Toxicology Program, 2004a) and involved intravenous and inhalation exposure of rats and mice to 1-*tert*-butoxypropan-2-ol (Dill *et al.*, 2004).

#### (*a*) *Oral administration*

Groups of three male Fischer 344 rats were administered a single oral dose of 3.8, 37.7 and 377.1 mg/kg bw [$^{14}$C]1-*tert*-butoxypropan-2-ol (which represented 0.1%, 1% and 10% of the $LD_{50}$) in water at 5 mL/kg and serial samples were collected for 72 h. The cumulative excretion profiles were similar at all dose levels and most of the radioactivity was recovered within 24 h. The predominant route of excretion (48–67% of the administered dose) was via the urine. The major urinary metabolite (23–52%) was the glucuronide conjugate of 1-*tert*-butoxypropan-2-ol (the highest percentage was recovered at the lowest dose); another significant urinary metabolite was the sulfate conjugate (6.7–13%; the highest percentage was recovered after at the highest dose). Unchanged 1-*tert*-butoxypropan-2-ol in urine accounted for less than 2% of the dose. Expiration of [$^{14}$C]carbon dioxide accounted for 22–26% of the dose, while elimination of exhaled volatile organic compounds was

negligible. Faecal elimination accounted for about 4% of the lowest dose but increased to 11% at the highest dose.

Since 26% of the radioactivity was detected as exhaled carbon dioxide, an alternative pathway of metabolism of 1-*tert*-butoxypropan-2-ol, similar to that of other propylene glycol ethers, may occur (Miller *et al*., 1984). This pathway could involve oxidation (*O*-dealkylation of 1-*tert*-butoxypropan-2-ol) to produce propylene glycol, which could be metabolized further to lactic acid and pyruvic acid, which may undergo tricarboxylic acid cycle conversion to carbon dioxide (Dill *et al*., 2004), as proposed in Figure 1. However, there are currently no empirical data to support the existence of this pathway for 1-*tert*-butoxypropan-2-ol.

Examination of the distribution of 1-*tert*-butoxypropan-2-ol in tissues after a 3.8-mg/kg dose showed that the five highest levels of radioactivity were in muscle (2.04 ± 0.18%), skin (1.39 ± 0.18%), fat (0.57 ± 0.12%), liver (0.39 ± 0.04%) and blood (0.28 ± 0.04%).

### (*b*) *Intravenous administration*

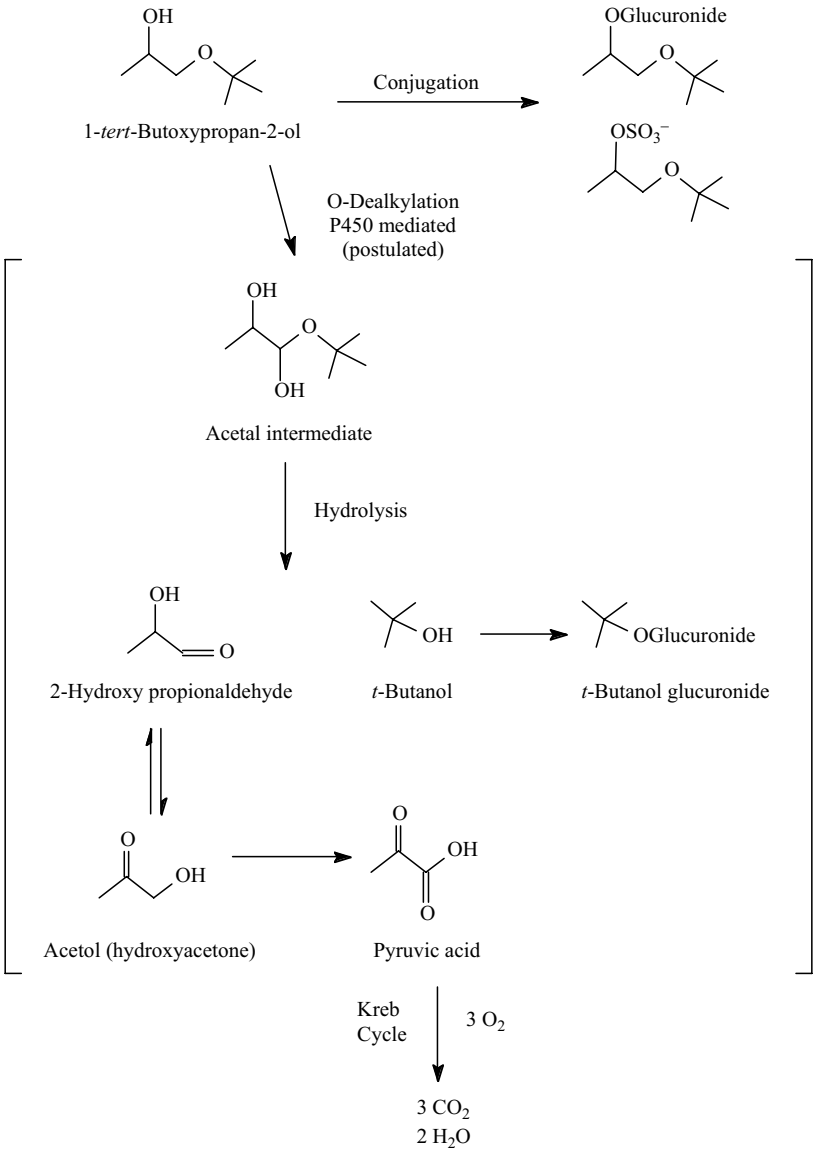
Pharmacokinetics were studied in Fischer 344 rats following an intravenous bolus dose of 37.8 mg/kg bw [$^{14}$C]1-*tert*-butoxypropan-2-ol. The mean plasma half-life was 16 min, the mean clearance was 25.1 mL/min/kg and the volume of distribution at steady state was 0.46 L/kg. Six hours after administration, 40% of the dose was recovered as the glucuronide conjugate in bile, although some of this appeared to be reabsorbed via enterohepatic circulation, as faecal elimination accounted for only 11% of the administered dose. The maximum rate of excretion was 30% of the dose per hour, which was reached between 0.5 and 1 h after administration (National Toxicology Program, 1994).

Fisher F344 rats and B6C3F$_1$ mice received a single intravenous injection of 15 or 200 mg/kg bw 1-*tert*-butoxypropan-2-ol. Serial blood samples were collected (three animals per species per sex per dose per time-point) after treatment for up to 12 h (rats) and 3 h (mice) and were analysed for 1-*tert*-butoxypropan-2-ol. Following the 15-mg/kg bw dose, the elimination half-life was 9.6 min in rats and 3.7 min in mice, but increased after the 200 mg/kg dose to 33.8 min and 9.4 min for rats and mice, respectively. The peak concentration of 1-*tert*-butoxypropan-2-ol in blood increased proportionally with dose (23 μg/g at 15 mg/kg and 284 μg/g at 200 mg/kg) and was not significantly different between the sexes in either species. Clearance in rats was slightly faster in males (30 and 11.5 mL/min/kg) than in females (23.2 and 8.8 mL/min/kg) after both doses (15 and 200 mg/kg) and was reduced with the higher dose; in mice, clearance was also reduced with the higher dose and was not significantly different between sexes (Dill *et al*., 2004).

### (*c*) *Dermal application*

In-vivo dermal absorption was determined in rats and mice using a skin-mounted trap that contained charcoal to maximize recovery of the dose. Following topical application of 4.7 mg/cm$^2$ [$^{14}$C]1-*tert*-butoxypropan-2-ol (to 8.4 cm$^2$ for rats and 0.8 cm$^2$ for mice), approximately 3% of the applied dose was absorbed in rats (approximately 2% of the dose was recovered in urine and 1% of the dose was exhaled as [$^{14}$C]carbon dioxide). The

**Figure 1. Proposed metabolic pathway for 1-*tert*-butoxypropan-2-ol**




Adapted from Dill *et al.* (2004)

majority of the dose was eliminated within 24 h and about 0.05% of the radioactivity was recovered from the site of application. For mice, a greater proportion of the dose was absorbed (7.8%); urinary radioactivity accounted for 2% and [$^{14}$C]carbon dioxide for 5% of the dose. The pattern of urinary metabolites was similar to that observed after oral administration (National Toxicology Program, 1994).

(*d*) *Inhalation exposure*

Rats and mice received a single 6-h whole-body inhalation exposure to 75, 300 or 1200 ppm [406, 1626 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol and serial blood samples were collected for up to 10 h (rats) and 4 h (mice) and analysed for the parent compound. In both species, blood concentrations of 1-*tert*-butoxypropan-2-ol declined rapidly after exposures to 75 or 300 ppm, but decreased more slowly after exposure to 1200 ppm. Although variable, the half-lives, estimated from the exposures to 75 ppm and 300 ppm, were approximately 20 min in rats and 5 min in mice and were not considered to differ within a species as a function of exposure concentration or sex. In rats, the peak blood concentration increased non-linearly with the exposure concentration in both sexes and was slightly but consistently higher (1.2–1.4-fold) in females (3.8, 23 and 368 μg/g at 75, 300 and 1200 ppm, respectively), which suggested saturation of metabolic clearance of 1-*tert*-butoxypropan-2-ol. Following exposure to 1200 ppm, the Michaelis-Menten constant ($K_m$, ~238 μg/g), maximum elimination rate ($V_{max}$, ~ 2.6 μg/g/min) and elimination rate constant ($K_e$, ~0.01 /min) were comparable between sexes. In mice, the peak blood concentration also increased non-linearly with the exposure concentration (around 1.5 μg/g and 16 μg/g for 75 and 300 ppm in both sexes) and, in addition to evidence of saturation of metabolism, there was a difference between the sexes at 1200 ppm (males, 547 μg/g; females, 800 μg/g). The $K_m$ (~98 μg/g), $V_{max}$ (~4.6 μg/g/min ) and $K_e$ (~0.04/min) were broadly comparable between sexes. Therefore, compared with rats, mice eliminated 1-*tert*-butoxypropan-2-ol from blood more rapidly (shorter half-life) and had higher efficiency (lower $K_m$) and capacity (higher $V_{max}$) for elimination (Dill *et al*., 2004).

In the same study (Dill *et al*., 2004), rats and mice were also exposed by inhalation (whole-body exposure) to 75, 300 or 1200 ppm [406, 1626 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for 14 (rats) or 16 (mice) weeks, as part of the National Toxicology Program 2-year inhalation study (National Toxicology Program, 2004a). At 2, 6, 14 (rats) and 16 (mice) weeks of exposure, a group of animals (three animals per species per sex per exposure concentration per time-point) was bled and samples were analysed for 1-*tert*-butoxypropan-2-ol. In rats, blood concentrations of 1-*tert*-butoxypropan-2-ol declined rapidly (half-life, approximately 10 min) with similar elimination rates between the groups exposed to 75 ppm and 300 ppm. For the group exposed to 1200 ppm, the initial elimination rate was slower but, after 3–4 h, was similar to that in the groups exposed to lower doses. The peak blood concentration increased in proportion to the dose between 75 ppm and 300 ppm but more than proportionally at 1200 ppm in both sexes. Effects of repeated exposures were insignificant from 2 to 14 weeks. In mice, blood concentrations of 1-*tert*-butoxypropan-2-ol declined rapidly after exposure to 75 and 300 ppm with

half-lives of approximately 5 min. Longer half-lives (approximately 14 min) were seen after exposure to 1200 ppm.

Animals in another group (10 animals per species per sex per exposure concentration) were placed in metabolism cages and urine samples were collected (on ice) for 16 h after exposure at 14 weeks (rats) and 16 weeks (mice) in the repeated inhalation study (Dill *et al.*, 2004). Samples were analysed for creatinine, 1-*tert*-butoxypropan-2-ol and its glucuronide and sulfate conjugates. In male and female rats, both the glucuronide and sulfate conjugates were detected in the urine; males excreted more total conjugates than females, which was consistent with a slower rate of clearance of 1-*tert*-butoxypropan-2-ol from the blood in females than in males. However, the conjugation pathway appeared to be saturated at the highest concentration, based on the lower ratio of total urinary conjugates to total exposure. The glucuronide conjugate was the predominant form, with levels 10–40-fold greater than those of the sulfate conjugate. The glucuronide:sulfate ratio decreased with increasing exposure concentration and duration in both sexes. In the earlier study by oral administration (National Toxicology Program, 1994), a relative decline in the glucuronide conjugate with increasing concentration of 1-*tert*-butoxypropan-2-ol was also noted. After exposure to 75 and 300 ppm, female rats excreted relatively more of the glucuronide conjugate than males, although this difference was not apparent after 1200 ppm.

As in rats, both the glucuronide and sulfate conjugates were detected in the urine of mice exposed to 1-*tert*-butoxypropan-2-ol, and the glucuronide was the predominant conjugate in both sexes (31–178-fold in males and 8–25-fold in females). The level of sulfate conjugates increased proportionally with exposure concentration in males exposed to 75 and 300 ppm, and more than proportionally after exposure to 1200 ppm; in females, the increase was proportional to exposure at all concentrations. In general, levels of glucuronide conjugate also increased with exposure concentration, but did not change with duration of exposure. Levels of the two conjugates combined increased more than proportionally with exposure concentration, and male mice excreted more than females. Contrary to the results in rats, male mice excreted relatively more of the glucuronide conjugate compared with the sulfate conjugate than female mice (Dill *et al.*, 2004).

The increase in total conjugated metabolites in the urine over time in both species may be indicative of induction of this metabolic pathway with prolonged exposure (Dill *et al*., 2004). Since metabolites that arise from the alternative potential pathway which involves oxidation (postulated cytochrome P450-mediated *O*-dealkylation that produces *tert*-butanol or acetol) were not measured, it was not possible to assess the potential for induction of this pathway. Induction of metabolism of 1-*tert*-butoxypropan-2-ol is consistent with the increases in relative liver weight observed in male and female rats and mice exposed for 13 weeks; these changes suggested proliferation of cellular organelles, perhaps those involved in xenobiotic metabolism (National Toxicology Program, 2004a).

### (*e*) *Prediction of metabolic pathways by computer modelling*

In view of the lack of empirical data, the Working Group investigated the probability that the oxidation pathway is important in the metabolism of 1-*tert*-butoxypropan-2-ol in

mammals. The expert system computer model META (Multicase, 2002) was used to predict the probable metabolic pathways for this substance and the potential relative contribution of each. Based on the model predictions, the principal pathway involves biotransformation of 1-*tert*-butoxypropan-2-ol via reductases to 3-(1,1-dimethylethoxy)-1-propene, which is subsequently converted by cytochrome P450 epoxidation of the double bond to the epoxide, *tert*-butyl glycidyl ether.[1] Conjugation with glucuronide is predicted to be the second most probable pathway, with lesser amounts of the sulfate conjugate being formed (consistent with the observations *in vivo*). Based on the model, the oxidation pathway described for other propylene glycol ethers represents only a minor pathway. However, other than conjugation with glucuronide and sulfate, these predicted pathways can only be speculative as they have not been investigated *in vivo*. In addition, elimination of a large proportion of the parent compound as expired carbon dioxide, as observed by Dill *et al.* (2004), was not predicted to be a significant removal process.

## 4.2 Toxic effects

### 4.2.1 *Humans*

No data were available to the Working Group.

### 4.2.2 *Experimental systems*

1-*tert*-Butoxypropan-2-ol has low acute toxicity in rats, with an $LD_{50}$ of 3771 mg/kg bw. Target organs following acute oral exposure to 2239–4467 mg/kg bw were the lungs, stomach, liver and kidneys (Boatman, 2001). In the only acute inhalation study identified, no deaths occurred in Sprague-Dawley rats exposed to 2680 mg/m$^3$ for 4 h. Mild extramedullary haematopoiesis was observed in the liver of both males and females. Occluded dermal exposure to 2 g/kg bw 1-*tert*-butoxypropan-2-ol did not cause death, clinical signs or effects on body weight in rabbits exposed for 24 h and observed for a subsequent 14 days, although signs of mild irritation were observed at the site of application in a primary skin irritation assay. However, 1-*tert*-butoxypropan-2-ol was severely irritating to the eyes of rabbits administered 0.1 mL of the neat liquid; slight irritation was observed when the substance was applied as a 20% aqueous solution (Boatman, 2001). [The Working Group

[1] The epoxide product, *tert*-butyl glycidyl ether, has been demonstrated to be mutagenic in *Salmonella* (Dabney, 1979; Connor *et al*., 1980; Canter *et al*., 1986; National Toxicology Program, 2004b) and to induce unscheduled DNA synthesis in cultured human lymphocytes (Frost & Legator, 1982). However, the root epoxide, glycidol, showed clear evidence of carcinogenicity in rats and mice of both sexes (National Toxicology Program, 1990a) and has been classified as a possible human carcinogen (IARC, 2000). In addition, the linear *n*-butyl glycidyl ether and allyl glycidyl ether were both genotoxic in in-vitro and in-vivo assays (Connor *et al*., 1980; Frost & Legator, 1982; Whorton *et al*., 1983; Canter *et al*., 1986; National Toxicology Program, 1990b, 2004c), while, for the latter substance, the National Toxicology Program (1990b) reported that there was equivocal evidence of carcinogenic activity in male rats and female mice, some evidence in male mice and no evidence in female rats.

noted the lack of detail in this secondary account of studies for which the original data are not publicly available.]

Groups of five male and five female Fischer 344/N rats and five male NBR rats were exposed by inhalation to 0, 75, 150, 300, 600 or 1200 ppm [406, 813, 1626, 3252 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for 16 days. NBR rats were used in this study in addition to Fischer 344/N rats because they do not produce hepatic $\alpha_{2u}$-globulin and, therefore, do not develop nephropathy associated with accumulation of this protein. No effects on body weight gain were observed in any exposed group, although relative liver weights were increased in male and female Fischer 344/N and male NBR rats exposed to the highest concentration and also in male Fischer 344/N rats exposed to 600 ppm; no histopathological changes were noted in the liver at any concentration of 1-*tert*-butoxypropan-2-ol. Relative kidney weights were also increased in male Fischer 344/N rats exposed to 600 ppm and above. Mild hyaline droplet accumulation in the kidneys was observed in all male but not in female Fischer 344/N rats or in NBR rats. In addition, increased cell proliferation was noted in the kidney of male Fischer 344/N rats at 1200 ppm (National Toxicology Program, 2004a).

In a concurrent short-term study, groups of five male and five female B6C3F$_1$ mice were exposed by inhalation to 0, 75, 150, 300, 600 or 1200 ppm [406, 813, 1626, 3252 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for 17 days. No clinical signs were noted at any exposure concentration. Although absolute and relative liver weights were significantly greater in females exposed to 300 ppm and above and in males exposed to 600 ppm and above compared with controls, no lesions related to exposure were observed in either sex of exposed mice (National Toxicology Program, 2004a).

In a subchronic study, groups of 10 male and 10 female Fischer 344/N rats were exposed by inhalation to 0, 75, 150, 300, 600 or 1200 ppm [406, 813, 1626, 3252 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for about 6 h per day on 5 days per week for 14 weeks. Additional groups of 10 males and 10 females exposed to the same concentrations were examined at 6 weeks for clinical pathology and renal effects, while renal effects were also examined in five additional male rats at 2 weeks. No effects on survival or body weight were observed in any exposed group. Relative kidney weights were increased in males exposed to all concentrations and in females exposed to 300 ppm or more. Signs of nephropathy, characteristic of that associated with the accumulation of $\alpha_{2u}$-globulin, included concentration-related increases in the severity or incidence of renal tubular hyaline droplet accumulation, cortical regeneration, medullary granular casts, increased $\alpha_{2u}$-globulin levels (confirmed by quantitation in kidney homogenates), cell proliferation and alterations in parameters of urinalysis (urine volume, glucose and protein concentrations and activities of aspartate aminotransferase, lactate dehydrogenase and *N*-acetyl-β-D-glucosaminidase) in males exposed to all concentrations of 1-*tert*-butoxypropan-2-ol at 12 and/or 14 weeks. The only changes in urinalysis parameters in female rats were increased activities of lactate dehydrogenase in those exposed to 150 ppm and above and of *N*-acetyl-β-D-glucosaminidase in those exposed to 600 ppm and above. Based on the occurrence of renal effects in female rats, although with less severity than those observed

in males, the authors speculated that a mechanism other than that related to $\alpha_{2u}$-globulin may be involved. Significant increases in relative liver weight were noted in males exposed to 150 ppm and above and in females exposed to 1200 ppm, which the authors hypothesized might be an adaptive response to altered liver function as demonstrated by transient increases in total bile acid concentrations that returned to control levels at termination of the study (14 weeks). Activities of alanine aminotransferase were decreased in females exposed to 150 ppm and above at day 23 but not at 14 weeks; this effect was observed in all exposed groups of males and persisted until the end of the study. No consistent effects on haematological parameters were observed that were considered to be related to exposure to 1-*tert*-butoxypropan-2-ol (National Toxicology Program, 2004a).

Male and female Fischer 344/N rats were exposed by inhalation to 28–709 ppm [152–3843 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for up to 13 weeks. Ten animals per exposure group were killed after 4 weeks of exposure, 13 weeks of exposure or 3 weeks after cessation of exposure. No effects on survival, body weight or haematological or clinical chemistry parameters were observed; similarly, no effects on bone marrow, blood or thymus were noted. Although increased weights of liver, kidneys and spleen were observed [concentrations at which these effects were observed were not specified], no histopathological alterations were found in these organs. The no-observed-adverse-effect level was reported to be 709 ppm (Boatman, 2001). [The Working Group noted the lack of detail in this secondary account of studies for which the original data are not publicly available.]

In the only available subchronic study in mice (National Toxicology Program, 2004a), groups of 10 male and 10 female B6C3F$_1$ mice were exposed by inhalation to 0, 75, 150, 300, 600 or 1200 ppm [406, 813, 1626, 3252 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for 14 weeks. Body-weight gain in male mice exposed to 150, 300 and 1200 ppm was significantly lower than that in controls. Liver weight was significantly increased in both sexes at 600 and 1200 ppm; this was accompanied by significant increases in the incidence of minimal-to-mild centrilobular hypertrophy (significant in males exposed to the two higher concentrations and in females only after the highest concentration). Minimal squamous metaplasia was noted in the nasal epithelium of male mice exposed to 1200 ppm 1-*tert*-butoxypropan-2-ol and in one female mouse in each of the groups exposed to 75 and 1200 ppm.

In a 2-year bioassay, groups of 50 male and 50 female Fischer 344/N rats were exposed by inhalation to 0, 75, 300 or 1200 ppm [406, 1626 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on 5 days per week for 104 weeks. The neoplastic effects observed in this study are reported in Section 3. Survival was decreased in males exposed to 300 ppm, but not to the highest concentration. Body weights were decreased in both sexes exposed to 1200 ppm. A concentration-related increase in the incidence of renal tubule hyperplasia was observed in males, which was significant at the two higher concentrations and was considered by the authors to be part of the continuum of progression to renal neoplasm. The severity of age-related nephropathy increased with increasing concentration of 1-*tert*-butoxypropan-2-ol in both male and female rats. The incidence, but not severity, of mild-

to-moderate hyaline droplet accumulation also increased with concentration in males and was significant for the two higher concentrations. It was noted that droplet accumulation was observed most frequently in male rats that died or were killed early during the study period. Other effects on the kidney observed in male rats included a significant increase in mineralization of the renal papilla with all concentrations and a significant increase in hyperplasia of the transitional epithelium of the renal pelvis with 1200 ppm. Similar to their speculations in the 14-week study (see above), the authors suggested that the nephropathy noted in female rats was not related to $\alpha_{2u}$-globulin. In the liver, a significant increase in the incidence of basophilic foci and clear-cell foci was observed in males and females, respectively, exposed to 1200 ppm. Significant concentration-related increases in the incidence and severity of hyaline degeneration of the olfactory epithelium occurred in both sexes of rats, together with significant increases in the incidence of submucosal gland dilatation in males exposed to 300 ppm and 1200 ppm and goblet-cell hyperplasia in males exposed to 1200 ppm; however, the authors considered these effects to be adaptive or protective responses to an irritant substance. Effects on the eye, including corneal opacity and corneal mineralization, were observed in females exposed to 1200 ppm, although the correlation between the two effects was poor (National Toxicology Program, 2004a).

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed by inhalation to 0, 75, 300 or 1200 ppm [406, 1626 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for approximately 6 h per day on 5 days per week for 104 weeks. A discussion of the observed neoplasms is presented in Section 3. Other than a slight decrease in body weight at the end of the study of females exposed to 1200 ppm, mean body weights were similar in exposed and control mice. In the liver, significant increases in the incidences of eosinophilic foci in both sexes exposed to 1200 ppm, in basophilic foci in males exposed to 300 ppm and in mixed-cell foci in males exposed to 1200 ppm were observed, and also an increase in the incidence of mild multinucleated hepatocytes in male mice exposed to the highest concentration. Male mice also had a significant increase in the incidence of forestomach inflammation after exposure to 300 and 1200 ppm (incidences of 2/48, 3/49, 9/50 and 9/50 at 0, 75, 300 and 1200 ppm, respectively) and of forestomach squamous epithelial hyperplasia with 300 ppm (incidences of 2/48, 5/49, 9/50 and 7/50 at 0, 75, 300 and 1200 ppm, respectively). However, the authors considered that these lesions were not related to exposure to 1-*tert*-butoxypropan-2-ol, as the severity of inflammation and hyperplasia was similar to that observed in controls. Similar to observations in the concurrent bioassay in rats (see above), effects on the cornea were observed in female mice exposed to 1200 ppm, and consisted of pale foci that corresponded partially with mild mineralization and, less frequently, inflammation, erosion and squamous hyperplasia.

## 4.3 Reproductive and developmental effects

### 4.3.1 *Humans*

No data were available to the Working Group.

### 4.3.2 *Experimental systems*

No published studies on the potential effects of exposure to 1-*tert*-butoxypropan-2-ol on reproductive function were identified.

In the 14-week inhalation studies conducted by the National Toxicology Program (2004a), groups of 10 male and 10 female Fischer 344/N rats and groups of 10 male and 10 female $B6C3F_1$ mice were exposed to 0, 300, 600 or 1200 ppm [1626, 3252 or 6504 mg/m$^3$] 1-*tert*-butoxypropan-2-ol. No effects on testes or epididymal weights or on sperm parameters, including sperm count and motility, were observed in either species. Similarly, no histopathological changes in reproductive organs occurred in either rats or mice. An increase in the length of the estrus cycle (mainly due to lengthened diestrus) was observed in female mice exposed to 1200 ppm, a concentration that was also associated with effects on the liver. No effects on the estrus cycle were observed in rats at any concentration.

Information on the developmental toxicity of 1-*tert*-butoxypropan-2-ol in experimental animals is sparse. Groups of 16 pregnant New Zealand white rabbits were exposed to airborne concentrations of 0, 229, 721 or 984 ppm [1241, 3908 or 5333 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on days 7–19 of gestation. No effects on behaviour, weight gain or haematological parameters were noted in the exposed dams, and no morphological effects were observed in fetuses at any concentration (Boatman, 2001). Similarly, no fetal toxicity or developmental effects were observed in groups of 25 pregnant CDF rats exposed to 0, 230, 726 or 990 ppm [1247, 3935 or 5366 mg/m$^3$] 1-*tert*-butoxypropan-2-ol for 6 h per day on days 6–15 of gestation. However, absolute and relative liver weights were significantly increased in dams exposed to the two higher concentrations and half of the dams exposed to 990 ppm were 'pale in appearance' during most of the exposure period (Boatman, 2001). [The Working Group noted the lack of detail, e.g. on maternal toxicity, in this secondary account of studies for which the original data are not publicly available.]

## 4.4 Genetic and related effects

### 4.4.1 *Humans*

No data were available to the Working group.

### 4.4.2 *Experimental systems* (see Table 3 for references and details)

The genotoxicity of 1-*tert*-butoxypropan-2-ol has been examined in a very limited number of assays, all of which were conducted as part of a study by the National Toxicology Program (2004a). The compound did not induce gene mutations in *Salmonella typhimurium* strains TA100, TA1535 or TA98 either in the absence or presence of an exogenous metabolic activation system (postmitochondrial supernatant) from the livers of Aroclor 1254-treated rats and hamsters. However, in strain TA97, 1-*tert*-butoxypropan-2-ol caused a concentration-related increase in mutant frequency in two repeat experiments in the same laboratory by factors of maximally 2.1 and 2.4, respectively, in the absence of

**Table 3. Genetic and related effects of 1-*tert*-butoxypropan-2-ol**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| *Salmonella typhimurium* TA100, TA1535, TA98, reverse mutation | – | –[c] | 10 000 µg/plate | National Toxicology Program (2004a) |
| *Salmonella typhimurium* TA1537, reverse mutation | – | NT | 10 000 µg/plate | National Toxicology Program (2004a) |
| *Salmonella typhimurium* TA97, reverse mutation | + | –[c] | 10 000 µg/plate | National Toxicology Program (2004a) |
| *Salmonella typhimurium* TA97, reverse mutation | + | –[c] | 1000 µg/plate | National Toxicology Program (2004a) |
| Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro* | – | –[d] | 1667 | National Toxicology Program (2004a) |
| Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro* | NT[e] | –[d] | 5000 | National Toxicology Program (2004a) |
| Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro* | – | –[d] | 5000 | National Toxicology Program (2004a) |
| Micronucleus formation, female B6C3F$_1$ mice, normochromatic erythrocytes in peripheral blood *in vivo* | (+) | | 1200 ppm, inhal. × 3 mo | National Toxicology Program (2004a) |
| Micronucleus formation, male B6C3F$_1$ mice, normochromatic erythrocytes in peripheral blood *in vivo* | – | | 1200 ppm, inhal. × 3 mo | National Toxicology Program (2004) |

[a] +, positive; –, negative; (+), weakly positive; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro studies, µg/mL; inhal., inhalation; mo, month
[c] From the livers of both Aroclor 1254-treated male Sprague-Dawley rats and Syrian hamsters
[d] From the livers of Aroclor 1254-treated male Sprague-Dawley rats
[e] Cytostatic effect without exogenous metabolic activation

an exogenous metabolic activation. No mutagenicity was observed in strain TA97 in the presence of metabolic activation. 1-*tert*-Butoxypropan-2-ol was not mutagenic in another strain that is responsive to frameshift mutations (TA1537) in the absence of an exogenous metabolic activation. This strain is generally regarded as being less sensitive to the action of frameshift mutagens than TA97.

Tests for the induction of sister chromatid exchange and structural chromosomal aberrations in Chinese hamster ovary (CHO) cells yielded negative results both in the absence and in the presence of an exogenous metabolic activation system from the livers of Aroclor 1254-treated rats.

The capacity of 1-*tert*-butoxypropan-2-ol to cause genotoxic effects *in vivo* was assessed by a micronucleus assay in male and female $B6C3F_1$ mice that had been exposed by inhalation to 75–1200 ppm [406–6504 mg/m$^3$] for 3 months. No increase in the frequency of micronucleated normochromatic erythrocytes was observed in males, and a weak increase was obtained in females. This increase showed a statistically significant trend, and the pairwise comparison of treated animals and the corresponding chamber controls yielded a statistically significant difference at the highest exposure concentration. The treatments did not affect the frequency of polychromatic erythrocytes in males or females, which indicated that the exposures had not been cytotoxic to the bone marrow (National Toxicology Program, 2004a).

### 4.5 Mechanistic considerations

The available data on the genotoxic potential of 1-*tert*-butoxypropan-2-ol are too limited to draw any sound conclusion regarding the role of potential mutagenic effects of the compound in the etiology of the neoplasms observed in animal studies. There is no plausible explanation for the weak but reproducible mutagenicity in *S. typhimurium* strain TA97, and it it is not clear whether there is a mechanistic link between the induction of mutations in this specific strain and tumour formation. According to the criteria of Ashby and Tennant (1991), the compound carries no structural alerts to genotoxicity, and various other glycol ethers (Zeiger *et al*., 1985, 1992; National Toxicology Program, 2000) (as well as closely related compounds such as *tert*-butylethyl ether (Zeiger *et al*., 1992)) were consistently non-mutagenic when tested in various *Salmonella* strains, including TA97. The increase in micronucleus formation in female mice exposed to 1-*tert*-butoxypropan-2-ol might appear to be more relevant to a potential link to tumour formation, but this finding should be viewed with caution, because the effect obtained was very weak, it was not observed in exposed males and, due to the design of the study, the assay was not repeated.

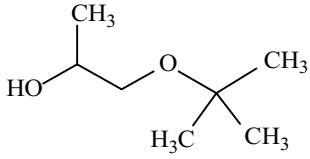
In 2-year studies carried out by the National Toxicology Program (2004a) on Fischer 344/N rats, the kidney was a target organ for the toxicity of 1-*tert*-butoxypropan-2-ol, and the results obtained indicate weak tumorigenicity of the compound in the kidneys of male, but not female rats (Doi *et al*., 2004; National Toxicology Program, 2004a). The renal lesions observed in male rats were characteristic of $\alpha_{2u}$-globulin-associated nephropathy, which suggests a possible mechanistic link between renal toxicity and

renal tumour response. However, neither the binding of 1-*tert*-butoxypropan-2-ol to $\alpha_{2u}$-globulin nor the formation of *tert*-butanol, a postulated metabolite of 1-*tert*-butoxypropan-2-ol (Dill *et al*., 2004) which has been reported to form a complex with $\alpha_{2u}$-globulin (Williams & Borghoff, 2001) and to cause $\alpha_{2u}$-globulin accumulation (Borghoff *et al*., 2001), were investigated in this study. However, although the binding of 1-*tert*-butoxypropan-2-ol to $\alpha_{2u}$-globulin has not been investigated, a structurally analogous alcohol compound, a metabolite of trimethylpentane, does bind to this protein and thereby induces nephrotoxicity in male Fischer 344 rats (Lock *et al*., 1987; Short *et al*., 1989). In a kidney initiation–promotion model, trimethylpentane has been shown to promote atypical cell foci and renal-cell tumours in male, but not female rats (Short *et al.*, 1989). Data from related investigations suggest that the tumour-promoting potential of trimethylpentane results from reversible binding of its metabolite, 2,4,4-trimethyl-2-pentanol, to $\alpha_{2u}$-globulin, which leads to decreased renal catabolism of this protein, chronic lysosomal overload, cell death and compensatory cell proliferation (Lock *et al.*, 1987; see also Capen *et al.*, 1999). This alcohol bears a structural resemblance to 1-*tert*-butoxypropan-2-ol (see Fig. 2).

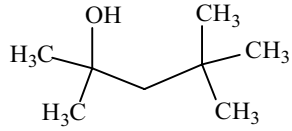
In addition to the renal effects in male rats exposed to 1-*tert*-butoxypropan-2-ol, a significant increase in the severity of age-related nephropathy was also observed in female rats exposed to 1200 ppm and, in the 14-week study, significant increases in absolute and relative kidney weights in females exposed to 300 ppm or higher concentrations; alterations in urinary chemistry parameters were also observed in female rats exposed to concentrations of 150 ppm 1-*tert*-butoxypropan-2-ol or higher. Although these changes were not accompanied by histopathological lesions and were less severe than those in male rats, exposure-related mechanisms of renal injury other than accumulation of $\alpha_{2u}$-globulin cannot be excluded (Doi *et al.*, 2004). The available data meet some but not all IARC criteria for a compound that induces renal toxicity and tumours via a mechanism associated with $\alpha_{2u}$-globulin accumulation in male rats (Capen *et al.*, 1999).

No mechanistic information related to the occurrence of increased incidences of liver neoplasms in male rats and both male and female mice exposed to 1-*tert*-butoxypropan-2-ol was available (Doi *et al*., 2004; National Toxicology Program, 2004a).

**Figure 2. Chemical structural resemblance of 1-tert-butoxypropan-2-ol and 2,4,4-trimethyl-2-pentanol**




1-*tert*-Butoxypropan-2-ol




2,4,4-Trimethyl-2-pentanol