3. Studies of Cancer in Experimental Animals

3.1 Inhalation

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3 F_1 mice, approximately 6 weeks of age, were exposed to 0, 75, 300 or 1200 ppm [0, 405, 1620 or 6480 mg/m^3] 1-*tert*-butoxypropan-2-ol (> 99% pure) vapour by whole-body exposure for 6 h per day on 5 days per week for 104 weeks. Complete necropsies were performed on all mice, when all organs and tissues were examined for macroscopic lesions, and all major tissues were examined microscopically. Exposure to 1-*tert*-butoxypropan-2-ol had no effect on survival of the mice. Survival rates in males were 35/50 (control), 40/50 (low-dose), 40/50 (mid-dose) and 37/50 (high-dose), and those in females were 39/50 (control), 36/50 (low-dose), 42/50 (mid-dose) and 39/50 (high-dose). Increases in the incidences of neoplastic and non-neoplastic lesions were observed in the livers of both male and female mice (Table 2). The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) occurred with positive trends in males and females ($p \leq 0.05$, Poly-3 test); the incidence of hepatoblastoma occurred with a positive trend in males ($p \leq 0.05$, Poly-3 test); and the incidences in the high-dose groups were significantly increased ($p \leq 0.001$, Poly-3 test). In males, the incidence of combined hepatocellular adenoma and carcinoma in the high-dose group exceeded that seen in historical controls (range, 50–68%). The incidence of hepatoblastomas in historical inhalation controls was 0/250 males and 0/248 females fed NTP-2000 diet. The overall incidence of hepatoblastomas in historical controls following exposure by all routes in all laboratories was 16/1159 (1.38%) males and 0/1152 females fed NTP-2000 diet. In high-dose females, the incidence of combined hepatocellular adenomas and carcinomas also exceeded that seen in historical controls (range, 22–37%). The incidences of hepatocellular foci were generally increased with exposure. The incidence of multinucleated hepatocytes in males exposed to 1200 ppm was significantly greater than that of controls. Multinucleated hepatocytes were randomly distributed; enlarged hepatocytes contained three or more nuclei. The severity of this change was generally mild and based on the number of multinucleated hepatocytes (Doi *et al*., 2004; National Toxicology Program, 2004a).

Table 2. Incidences[a] of liver lesions in B6C3F$_1$ mice in the 2-year inhalation study of 1-*tert*-butoxypropan-2-ol

Type of tumour or lesion	Controls	75 ppm	300 ppm	1200 ppm
Males				
No. examined	50	49	50	50
Hepatocellular adenoma	18[b]	23	26	36*
Hepatocellular carcinoma	9	8	13	11
Hepatocellular adenoma or carcinoma	25[b]	26	33	41*
Hepatoblastoma	0[b]	0	1	5*
Eosinophilic focus	9	14	11	29*
Hepatocyte, multinucleated	27 (1.0)[c]	23 (1.0)	24 (1.0)	46* (1.8)
Females				
No. examined	49	50	50	49
Hepatocellular adenoma	14[b]	8	10	37*
Hepatocellular carcinoma	4	8	7	10
Hepatocellular adenoma or carcinoma	18[b]	14	16	41*
Hepatoblastoma	0	0	0	2
Eosinophilic focus	11	10	9	27*

From Doi *et al.* (2004); National Toxicology Program (2004a)
* Significantly different ($p \leq 0.05$) from concurrent controls by the Poly-3 test
[a] Number of animals with lesion
[b] Overall exposure-related trend ($p \leq 0.05$) by the Poly-3 test
[c] Average severity grade of lesions in affected animals is depicted in parentheses: 1, minimal; 2, mild; 3, moderate; 4, marked.

3.1.2 *Rat*

Groups of 50 male and 50 female Fisher 344/N rats, approximately 6 weeks of age, were exposed to 0, 75, 300 or 1200 ppm [0, 405, 1620 or 6480 mg/m^3] 1-*tert*-butoxypropan-2-ol (> 99% pure) vapour by whole-body exposure for 6 h per day on 5 days per week for 104 weeks. Complete necropsies were performed on all rats, when all organs and tissues were examined for macroscopic lesions, and all major tissues were examined microscopically. Kidneys of male rats were step-sectioned to obtain three to four additional sections. Survival rates in males were 27/50 (control), 29/50 (low-dose), 16/50 (mid-dose) and 22/50 (high-dose); those in females were 33/50 (control), 34/50 (low-dose), 28/50 (mid-dose) and 36/50 (high-dose). No increase in the incidence of tumours was observed in females. Marginal increases in the incidences of kidney and liver tumours were observed in exposed males. The incidence of renal tubule adenomas in exposed males was marginally increased. One renal tubule carcinoma also occurred in the high-dose group. The combined incidences of these tumours were 1/50 (control), 2/50 (low-dose), 5/49 (mid-dose) and 5/50 (high-dose) (not statistically significant by pair-wise comparison and trend test). Historically, no more than one kidney neoplasm has been observed in male control rats fed NTP-2000 diet in National Toxicology Program inhalation studies, and the inci-

dences observed in the current study were greater than the National Toxicology Program historical control range (0–2%). The incidence of hepatocellular adenoma occurred with a positive trend ($p = 0.022$, Poly-3 test) in male rats, and the incidence in the high-dose group exceeded the historical range in controls fed NTP-2000 diet (0–6%) in the National Toxicology Program database. The incidence of liver adenoma in males was 3/50 (control), 0/50 (low-dose), 2/49 (mid-dose) and 6/50 (high-dose) (not statistically significant by pairwise comparison). No hepatocellular carcinomas were observed (Doi *et al*., 2004; National Toxicology Program, 2004a).