Carcinogenesis Studies of Tetrahydrofuran Vapors in Rats and Mice

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Tetrahydrofuran (THF) is a widely used industrial solvent and was selected for carcinogenesis studies by the National Toxicology Program (NTP) because of its potential for widespread occupational exposure in humans and a lack of information on animal toxicity and carcinogenicity. Groups of 50 male and 50 female F344/N rats and B6C3F1 mice were exposed to 0, 200, 600, or 1800 ppm THF by inhalation, 6 h per day, 5 days per week, for 105 weeks. Survival and mean body weights of male and female rats exposed to THF were comparable to that of the controls. No clinical findings or nonneoplastic lesions related to THF exposure were observed in male or female rats. The incidences of renal tubule epithelial adenoma or carcinoma (combined) in exposed male rats occurred with a positive trend, and in males exposed to 600 and 1800 ppm exceeded the historical range for controls in 2-year NTP inhalation studies. There were no other nonneoplastic lesions related to THF exposure observed in male or female rats. After week 36, the survival of male mice exposed to 1800 ppm was significantly lower than that of the controls. Mean body weights of male and female mice exposed to THF were similar to those of the controls throughout the study. Male mice exposed to 1800 ppm were observed in a state of narcosis during and up to 1 h after the exposure periods. Nonneoplastic lesions related to THF exposure were not observed in male or female mice. The nonneoplastic lesions related to THF exposure were seen in female mice only. In female mice exposed to 1800 ppm, the incidences of hepatocellular neoplasms were significantly greater than those in the controls. In conclusion, there was some evidence of carcinogenic activity of THF in male F344/N rats due to increased incidences of adenoma or carcinoma (combined) of the kidney at the 600 and 1800 ppm exposure levels. There was clear evidence of carcinogenic activity in male B6C3F1 mice based on increased incidences of hepatocellular neoplasms at the 1800 ppm exposure level. THF was not carcinogenic in female rats or male mice exposed at 200, 600, or 1800 ppm.

Tetrahydrofuran (THF) is a colorless, volatile liquid with an ethereal odor and a pungent taste. Human exposure is primarily during its use as a solvent for resins, adhesives, printer’s ink, and coatings. Approximately 90,000 workers in 3000 plants are exposed from industries related to chemical and allied products, electric, gas, and sanitary services. Electricians, agricultural and biological technicians, electric power linemen, and cable linemen are potentially exposed to THF. THF vapors may cause irritation of the mucous membranes, respiratory system, and skin. It is a strong narcotic. Acute toxicity could lead to narcosis, muscular hypotonia, and loss of corneal reflexes followed by coma and death (HSDB, 1996). Gosselin (1976) assigned a toxicity rating of “very toxic” for the THF with a probable oral lethal dose in humans of 50–500 mg/kg.

Katahira et al. (1982a) studied the acute toxicity of 20% THF in olive oil by intraperitoneal injection in rats and mice. The LD50 was 1900 mg/kg in rats and 2500 mg/kg in mice. The LC50 was estimated to be 21,000 ppm in rats exposed to THF for 3 h by inhalation route of exposure. Rats exposed to 5000 ppm had marked edema or opacity of the cornea, salivation, and discharge or bleeding in nasal mucosa. A cataleptic posture, coma, and clonic muscle spasms indicating CNS toxicity were also observed. Irritation of the upper respiratory tract and some injury to the liver and kidney were observed in a number of rats when exposed at a concentration of greater than 3000 ppm for 8 h daily for 20 days. A concentration of 25,000 ppm was required to produce anesthesia in dogs and mice (ACGIH, 1986).

Male Sprague–Dawley rats were exposed to 0, 100, 200, 1000, or 5000 ppm by inhalation for 4 h/daily, 5 days/week for a total of 12 weeks (Katahira, 1982b). Symptoms of toxicity (effects on CNS) were observed in animals exposed to 1000 or 5000 ppm; symptoms were of greater intensity initially at 5000 ppm; however, animals appeared to develop tolerance to THF since the magnitude of effects was reduced. Aspartate aminotransferase (AST), cholinesterase, and blood sugar values were increased in rats exposed to 1000 or 5000 ppm.

Subchronic toxicity of THF vapors in rats and mice was studied by the NTP (Chhabra et al., 1990). Groups of 10 rats and mice of each sex were administered THF vapors by whole-body inhalation for 13 weeks at exposure concentrations of 0, 66, 200, 600, 1800, or 5000 ppm. Clinical signs of central nervous system toxicity were observed in both rats and mice at high dose levels. Rats of both sexes exposed to 5000 ppm were ataxic and mice of both sexes exposed to 1800 or 5000 ppm appeared to be in a state of narcosis.
There were no exposure-related gross necropsy findings in rats or mice. At 5000 ppm, decreases in thymic and spleen weights in rats and mice of both sexes and increases in liver weights in both sexes of mice and female rats were observed. A minimal to mild centrilobular hepatocytomegaly occurred in male and female mice exposed to 5000 ppm THF.

In this paper, the results of NTP carcinogenicity studies on THF are summarized. Further details of the toxicity and chronic carcinogenicity studies on THF are provided in a technical report prepared by the NTP (NTP, 1996).

**MATERIALS AND METHODS**

**Animals.** Male and female F344/N rats and B6C3F1 mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). Rats and mice were quarantined for 14 or 15 days before the beginning of the studies. Five male and five female rats and mice were selected for parasite evaluation and gross observation for disease. Serology samples were collected for viral screening from five sentinel animals/species/sex at 6, 12, and 18 months and at termination of the studies. There were no clinical findings or histopathologic changes attributable to animal diseases monitored at the beginning and during the course of studies.

**Tetrahydrofuran.** THF (CAS 109-99-9) was obtained from ChemCen-Technology (Kansas City, MO). The purity of lots was determined by elemental analyses, Karl Fischer water analyses, and gas chromatography. Elemental analyses for hydrogen and carbon were generally in agreement with the theoretical values for THF. Karl Fischer water analysis indicated 0.06 to 0.1% water. Peroxide concentrations were no greater than 3 ppm. The overall purity was determined to be approximately 99%.

**Vapor generation and exposure system.** THF vapor was generated with a rotary evaporation system (Büchi Rotavapor, Model EL-131S, Büchi Laboratoriums Technik AG, Flavil, Switzerland). From the condensing column of the rotary evaporator, the vapor entered a short distribution manifold from which individual delivery lines carried metered amounts of vapor to each exposure chamber. When equilibrium was reached, each valve was opened to allow the flow of vapor into the chamber. At each chamber location, the vapor was injected into the chamber inlet duct where it was further diluted with charcoal- and HEPA-filtered chamber air to achieve the desired exposure concentration. Stainless-steel chambers designed at Battelle Northwest Laboratories were used for all studies (Hazleton 2000, Aberdeen, MD). The chamber was designed so that uniform vapor concentrations could be maintained throughout the chamber when catch pans were in position. A small particle detector (Type CN, Gardner Associates, Schenectady, NY) was used with and without animals in the exposure chambers to ensure that THF vapor and not aerosol was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm³) were detected.

The chamber concentrations of THF were monitored using an on-line gas chromatograph with a flame ionization detector and a 1% SP-1000 on 60/80 Carbopack B nickel column. Samples were drawn and analyzed from each exposure chamber, the control chamber, the exposure suite, an on-line standard, and filtered air blank approximately every 30 min using a 12-port sample valve. Summaries of the chamber concentrations attained during the exposures are provided in Table 1. No degradation products at concentrations could be maintained throughout the chamber when catch pans were opened to allow the flow of vapor into the chamber. At each chamber location, the vapor was injected into the chamber inlet duct where it was further diluted with charcoal- and HEPA-filtered chamber air to achieve the desired exposure concentration. Stainless-steel chambers designed at Battelle Northwest Laboratories were used for all studies (Hazleton 2000, Aberdeen, MD). The chamber was designed so that uniform vapor concentrations could be maintained throughout the chamber when catch pans were in position. A small particle detector (Type CN, Gardner Associates, Schenectady, NY) was used with and without animals in the exposure chambers to ensure that THF vapor and not aerosol was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm³) were detected.

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**Experimental design.** Rats and mice were approximately 6 weeks old at the beginning of the studies. Rats and mice were housed individually. NIH-07 open formula pelleted feed (Zeigler Brothers, Inc., Gardners, PA) and tap water were available ad libitum except during exposure periods. Techsorb bedding (Shepherd Specialty Papers, Inc., Kalamazoo, MI) was used and changed during nonexposure periods, 7 days per week. Cages and racks were rotated within inhalation chamber weekly. Groups of 50 male and 50 female rats and mice were exposed to THF at concentrations of 0, 200, 600, and 1800 ppm by inhalation, 6 h plus 17 h (12 min) per day, 5 days per week, for 105 weeks.

**Clinical examinations and pathology.** All animals were observed twice daily. Clinical findings and body weights were recorded weekly for 12 weeks (rats) or 13 weeks (mice), monthly thereafter through week 91 (rats) or week 92 (mice), and then every 2 weeks until the end of the studies. Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on all animals.

**Alpha 2μ-microglobulin evaluation.** Immunohistochemical staining was performed on sections of kidney from control and 1800 ppm male rats from the 13-week study using a Vectastain Elite ABC anti-mouse IgG kit (Vector Labs, Burlingame, CA) and DAB chromagen (Prescott-Mathews et al., 1997; Burnett et al., 1989). A mouse monoclonal antibody (Hazleton Biotechnologies Co., Vienna, VA) was used as the primary antibody at a dilution of 1:800. Kidney sections were graded subjectively (with no knowledge of treatment group) on a scale of 1-4 based on the amount of positively staining protein droplet (α 2μ-microglobulin) accumulation in the cytoplasm of renal tubules and the character of the droplet aggregates (fine and dispersed and/or coarse-aggregated with crystalline forms).

**Analysis of neoplasm incidences.** The primary statistical method used for comparison of tumor incidence was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from unrelated cause and thus did not affect the risk of death (Dinse and Haseman, 1986).

**RESULTS**

**Rats**

**Survival, body weights, and clinical observations.** Survival of male and female rats exposed to THF was similar
There were no other neoplastic lesions present in male or female rats. Neoplasms were not observed in exposed female rats. Also, nonneoplastic lesions related to THF exposure were not observed in male or female rats related to THF exposure. Loss of the tubular architecture and/or large sheets or nests of atypical cells and invariably contained areas in which there were multiple solid nests of polygonal, basophilic cells separated by a delicate vascular stroma. The neoplastic cells showed mild cellular and nuclear pleomorphism and atypia, and occasionally mitotic cells were evident. Renal tubule carcinomas were well-demarcated nodular masses generally larger than adenomas. Carcinomas were composed of karyomegaly atypical cells and invariably contained areas in which there was loss of the tubular architecture and/or large sheets or nests of atypical cells containing large vacuoles. Renal tubule neoplasms were not observed in exposed female rats. There were no other neoplastic lesions present in male or female rats related to THF exposure. Also, nonneoplastic lesions related to THF exposure were not observed in male or female rats.

**Table 2**

Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male Rats Exposed to Tetrahydrofuran for Two Years

<table>
<thead>
<tr>
<th>Renal tubule</th>
<th>Control</th>
<th>200 ppm</th>
<th>600 ppm</th>
<th>1800 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number examined</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Nephropathy, chronic</td>
<td>48* (3.0)*</td>
<td>50 (2.9)</td>
<td>50 (3.1)</td>
<td>50 (3.0)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>7 (3.8)</td>
<td>5 (3.6)</td>
<td>6 (3.3)</td>
<td>7 (3.3)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
<td>4/50 (8%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adenoma/carcinoma</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
<td>4/50 (8%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Logistic Regression Test</td>
<td>$p = 0.037$</td>
<td>$p = 0.602$</td>
<td>$p = 0.159$</td>
<td>$p = 0.065$</td>
</tr>
</tbody>
</table>

* Number of animals with lesions.

* Average severity of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.

* Historical incidence for 2-year inhalation studies with chamber controls: 6652 (0.9 ± 1.3%).

* In the control column are the $p$ values associated with the trend test. In the exposed group column are the $p$ values corresponding to the pairwise comparison between the controls and that of exposed group.

Alpha 2μ-microglobulin evaluation. There were major observable differences between the controls and 1800 ppm rats. The protein droplets in control rats were finer and more densely and diffusely distributed in the cytoplasm of tubular epithelial cells in the outer cortex, whereas in 1800 ppm rats the droplets were more coarse and concentrated in scattered foci in the outer cortex. However, the average severity grades for droplet accumulation did not differ significantly between the controls (2.6) and 1800 ppm dose group (2.8).

**Mice**

Survival, body weights, and clinical observations. After week 36, the survival of male mice exposed to 1800 ppm was significantly lower than that of the controls; the average lifespan (456 days) of male mice in the 1800 ppm exposure group was significantly less than that of the controls (689 days). Survival of males exposed to 200 or 600 ppm and of all exposed female groups was similar to that of the controls. Mean body weights of male and female mice exposed to THF were similar to those of the controls throughout the study (data not shown). No clinical findings related to THF exposure were observed in male or female rats.

Neoplastic lesions. The incidences of renal tubule epithelial adenoma were increased in the 600 and 1800 ppm males (Table 2). In addition, two male rats receiving 1800 ppm had renal tubule epithelial carcinomas. Although not statistically significant, the incidences of adenoma or carcinoma (combined) in the 600 and 1800 ppm males exceeded the historical range for controls in 2-year NTP inhalation studies (Table 2). Renal tubule epithelial adenoma or carcinoma (combined) occurred with a positive trend in male rats. The majority of the adenomas were well-circumscribed nodular masses (usually larger than 5 or more tubule diameters), some of which were detected grossly. Morphologically, the renal tubule neoplasms observed in treated rats were similar to those that developed spontaneously in control mice. There were major observable differences between the controls and 1800 ppm rats. The protein droplets in control rats were finer and more densely and diffusely distributed in the cytoplasm of tubular epithelial cells in the outer cortex, whereas in 1800 ppm rats the droplets were more coarse and concentrated in scattered foci in the outer cortex. However, the average severity grades for droplet accumulation did not differ significantly between the controls (2.6) and 1800 ppm dose group (2.8).

Neoplastic lesions. The incidences of hepatocellular neoplasms (adenoma and carcinoma) in female mice exposed to 1800 ppm were significantly greater than those in the controls (Table 3); in addition, the incidences of multiple hepatocellular neoplasms were increased in female mice exposed to 1800 ppm. The incidences of combined hepatocellular neoplasms in female mice exposed to 1800 ppm significantly exceeded the historical range for controls in 2-year NTP inhalation studies (Table 3). Morphologically, the hepatocellular neoplasms observed in treated mice were similar to those that developed spontaneously in control mice.
Hepatocellular adenomas were nodular lesions composed of eosinophilic to basophilic cells characterized by mild nuclear atypia and increased nuclear/cytoplasmic ratio. Adenomas were well-demarcated from the normal adjacent parenchyma by the sharp abutment of the neoplastic cells with and compression of normal hepatocytes along most borders of the mass. Within the mass, the normal hepatic architecture was disrupted. Hepatocellular carcinomas were large, irregular, poorly to well-demarcated masses composed of large pleomorphic atypical cells which were frequently arranged in trabecular patterns in some areas. The incidences of hepatocellular neoplasms in exposed male mice were not significantly different from those in the controls.

**Nonneoplastic lesions.** Male mice exposed to 1800 ppm had significantly greater incidences of nonneoplastic lesions of the urogenital tract than those in the controls. These lesions (which occurred primarily among the 26 animals dying during the first 52 weeks of the study) included suppurative inflammation of the kidney, urinary bladder, prostate gland, and preputial skin. The character of inflammatory lesions suggested an ascending bacterial infection. Prolonged wetting of the preputial fur during exposure-related narcosis may have predisposed these animals to a preputial and, subsequently, an ascending urogenital tract bacterial infection resulting in moribundity and ultimately death. Therefore, these lesions were not considered to be related to THF exposure.

There were no other nonneoplastic lesions related to THF exposure observed in male or female mice.

**DISCUSSION**

In previously published NTP 13-week toxicity studies in rats and mice, animals were exposed to THF at 66, 200, 600, 1800, or 5000 ppm (Chhabra et al. 1990). THF was found to cause reversible CNS depression in rats and mice. Male and female mice exposed to 1800 or 5000 ppm were observed in a state of narcosis (described by stupor) during exposure periods. Mice exposed to 1800 ppm were fully awake and alert immediately after exposure; however, mice exposed to 5000 ppm required up to 2 h for recovery. The animals had developed some tolerance to this effect by the end of the 13-week studies. For 2-year carcinogenesis studies, THF concentrations of 200, 600, and 1800 ppm were selected for both rats and mice. It was predicted that in the 2-year study, mice in the 1800 ppm group might develop a complete tolerance to the sedative effect of THF, and therefore sedation might not have an adverse effect on survival related to THF exposure. The prediction was clearly incorrect for male mice, and 26 mice in the 1800 ppm group died during the first year of the study from sequelae associated with narcosis. A number of male mice exposed to 1800 ppm were observed to be in a state of narcosis during and up to 1 h after exposures. Prolonged wetting of the preputial fur during exposure-induced narcosis may have predisposed these animals to a preputial and, subsequently, an ascending bacterial infection of the urogenital tract that resulted in moribundity and ultimately death. Therefore, the highest exposure concentration (1800 ppm) selected for male mice in this study exceeded the maximum tolerated dose (MTD). The survival of exposed groups of male and female rats and of female mice was similar to that of the control groups. The mean body weights of exposed groups of rats and mice were similar to those of the control groups throughout the study.

The increased incidence of renal tubular neoplasms in male rats exposed to THF was considered to be related to the administration of THF. Several factors predicted the
assertion of a carcinogenic effect in the male rat kidney. First, there were increased incidences of relatively uncommon spontaneous renal neoplasms (less than 1% in NTP 2-year studies) in rats exposed to 600 ppm (8%) or 1800 ppm (10%). Second, although they were not statistically significantly greater than those of the controls but were increased in a positive trend, the incidences of renal neoplasms exceeded the historical control values for inhalation studies (range, 0–4%; rate, 0.9 ± 1.3%). Finally, there was a lack of a chemical-related increase in the incidence and/or severity of age-related degenerative renal disease (chronic progressive nephropathy) in exposed male rats. Marginal to slight increases in renal tubule neoplasms often accompany chemical-related exacerbation of nephropathy in rats. Therefore, it is often difficult to determine if neoplasms of the renal tubule develop as a direct effect of chemical administration or as an indirect effect secondary to the exacerbated nephropathy. The apparent lack of such a concentration-related increase in the incidence and/or severity of nephropathy in exposed male rats in this study eliminates this as a possible mechanism.

In female mice exposed to 1800 ppm THF, the incidence and multiplicity of liver neoplasms were significantly greater than those of the controls. The increases in the incidences of liver neoplasms in the 200 and 600 ppm exposure groups were not statistically significant, but the trend test was positive. There was no indication of an increase in the incidences of hepatocellular neoplasms in male mice. There are several possible reasons for this. The male mice may not have been sensitive enough to exhibit the THF-induced carcinogenic effect, due to a very high incidence of liver neoplasms in the controls (70%) and to the lower survival rate of high-dose mice. Alternatively, there may be an inherent sex difference in THF-induced liver neoplasms in mice.

The liver was also one of the major sites of carcinogenicity identified for furan and 1,4-dioxane, chemicals structurally related to THF (IARC, 1976). 1,4-Dioxane, a cyclic ether solvent structurally related to THF, was carcinogenic in rats and guinea pigs when administered by gavage. 1,4-Dioxane produced malignant neoplasms in the liver and nasal cavity in rats and neoplasms of the liver and the gall bladder in guinea pigs (IARC, 1976). It was also a promoter in a two-stage skin carcinogenesis study in mice.

The differences in THF-induced neoplasms between rats and mice and between males and females could be due to differences in the metabolism and disposition of THF. There is very little information in the literature on the toxicokinetics and cellular toxicity on this widely used solvent. Based on the limited information available from genotoxicity studies, the carcinogenic activity of THF observed in the current studies is most likely through nongenotoxic modes of action. THF was not mutagenic in a variety of in vitro and in vivo mutagenic assays (Mortelmans et al., 1986; Galloway et al., 1987; NTP, 1996). In some studies, the development of renal neoplasms in male rats has been related to the presence of degenerative hyaline droplet (α 2-μ-globulin) nephropathy, a species- and sex-specific lesion possibly mediated through nongenetic modes of action (Swenberg, 1993). Kawata and Ito (1984) reported the kidney as one of the target organs of THF toxicity. In that study, marginal increases in protein casts in the lumen of the kidney tubules and hyaline droplets in the cytoplasm of the tubular epithelial cells were observed after THF exposure by inhalation for 12 weeks at 3000 ppm. However, no such THF-related nonneoplastic lesions were observed in the kidneys of male rats in the 13-week or 2-year NTP studies.

Based on the 13-week inhalation toxicity (Chhabra et al., 1990) study in mice and on developmental toxicity studies (Mast et al., 1992), the no-observable-adverse-affect level (NOAEL) for THF-induced nonneoplastic lesions was 600 ppm in mice. In rats, the NOAEL for nonneoplastic lesions was 1800 ppm based on the 13-week inhalation toxicity studies (Chhabra et al., 1990).

In conclusion, there was some evidence of carcinogenic activity of THF in male F344/N rats based on increased incidences of adenoma or carcinoma (combined) of the kidney at the 600 and 1800 ppm exposure concentrations. There was clear evidence of carcinogenic activity in female B6C3F1 mice due to increased incidences of hepatocellular neoplasms at the 1800 ppm exposure concentration. THF was not carcinogenic in female rats and male mice.

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.


