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TOXICOLOGICAL REVIEW

OF

Tetrahydrofuran

(CAS No. **109-99-9**)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

August 2007

NOTICE

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U.S. Environmental Protection Agency
Washington, DC

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(CAS No. 109-99-9)

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ABBREVIATIONS AND ACRONYMS

ABT	1-aminobenzotriazole
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike Information Criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATH	atypical tubule hyperplasia
ATPase	adenosine triphosphatase
AUC	area under the curve
BASF	Badische Anilin- und Sodafabrik
BMC	benchmark concentration
BMCL	95% lower bound on the BMC
BMD	benchmark dose
BMDL	95% lower bound on the BMD
BMDS	BMD software
BMR	benchmark response
BPE	benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide
BrdU	5-bromo-2-deoxyuridine
CASRN	Chemical Abstracts Services Registry Number
Cl	clearance
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
dUTP	deoxyuridine triphosphate
EEG	electroencephalogram
EPA	U.S. Environmental Protection Agency
EROD	ethoxyresorufin-O-deethylase
FOB	functional observational battery
GABA	γ-aminobutyric acid
GBL	γ-butyrolactone
GGT	γ-glutamyl transferase
GHB	γ-hydroxybutyric acid
GI	gastrointestinal
GJIC	gap junctional intercellular communication
HEC	human equivalent concentraion
i.p.	intraperitoneal

IRIS	Integrated Risk Information System
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTP	National Toxicology Program
PBTK	physiologically based toxicokinetic
PCNA	proliferating cell nuclear antigen
PI_{50}	50% reduction of cell protein content
PND	postnatal day
PROD	pentoxyresorufin-O-dependentylase
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RGDR	regional gas dose ratio
THF	tetrahydrofuran
TUNEL	terminal deoxynucleotidyl dUTP nick-end-labeling staining
UF	uncertainty factor
VOC	volatile organic compound

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to tetrahydrofuran. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of tetrahydrofuran.

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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This document has been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information, and EPA's regional offices.

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of tetrahydrofuran. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values may also be derived for acute ($\leq$24 hours), short-term (up to 30 days), and subchronic (up to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is an upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water or per $\mu g/m^3$ air breathed. Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

Development of these hazard identification and dose-response assessments for tetrahydrofuran has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel reports that were used in the development of this assessment may include the following: *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a),

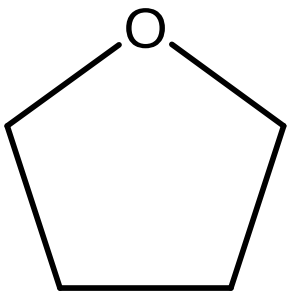
Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006, 2000a, 1998b), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the Chemical Abstracts Services Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through August 2006.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENT

Tetrahydrofuran (THF) is a synthesized organic compound that is not found in the natural environment (American Conference of Governmental Industrial Hygienists [ACGIH], 2001). It is a colorless, volatile liquid with an ethereal or acetone-like smell and is miscible in water and most organic solvents. Table 2-1 summarizes the physical and chemical properties of THF. THF is highly flammable. Upon contact with air, THF can decompose into explosive peroxides and carbon monoxide.

Table 2-1. Chemical and physical properties of tetrahydrofuran

CAS Registry Number	109-99-9	Verschueren (2001)
Synonym(s)	THF; diethyleneoxide; tetramethyleneoxide; 1,4 -epoxy butane; furanidine; oxacyclopentane	Verschueren (2001)
Melting point, °C	–108.5	Verschueren (2001)
Boiling point, °C	65/66	Verschueren (2001)
Vapor pressure, atm at 20°C	0.173	Verschueren (2001)
Density, at 20°C relative to the density of H_2O at 4°C	0.89	Verschueren (2001)
Flashpoint (closed cup)	–17 to –21.5°C	BASF (1993)[a]
Water solubility	Miscible	NIOSH (1997)[a]
Log K_{OW}	0.46	SRC (2001)[a]
Odor threshold	2 ppm–7.4 ppm 60–150 mg/m^3	ACGIH (2001); RIVM (2001)[a]
Molecular weight	72.10	Verschueren (2001)
Conversion factors	1 ppm = 2.95 mg/m^3	NIOSH (1997)
Empirical formula	C_4H_8O	Verschueren (2001)
Chemical structure		Verschueren (2001)

[a]BASF = Badische Anilin- und Sodafabrik; NIOSH = National Institute for Occupational Safety and Health; RIVM = National Institute for Public Health and the Environment (Netherlands); SRC = Syracuse Research Corporation.

THF is used as a solvent for polyvinyl chlorides, vinylidene chloride polymers, and natural and synthetic resins (particularly vinyls) and in topcoating solutions, polymer coatings, cellophane, protective coatings, adhesives, magnetic strips, and printing inks. It is also used for

Grignard and metal hydride reactions. THF is used as an intermediate in chemical synthesis. For example, it is used in the preparation of chemicals, including adipic acid, butadiene, acrylic acid, butyrolactone, succinic acid, 1,4-butanediol diacetate, motor fuels, vitamins, hormones, pharmaceuticals, synthetic perfumes, organometallic compounds, and insecticides. It is also used in the manufacture of polytetramethylene ether glycol, polyurethane elastomers, and elastic polymers. THF can be used in the fabrication of materials for food packaging, transport, and storage. When THF is used in food processing, it can be an indirect food additive (National Toxicology Program [NTP], 1998).

Potential exposures to humans result from anthropogenic sources, primarily from occupational exposures related to THF's use as a solvent for resins, adhesives, printers' ink, and coatings. Exposure to THF is primarily through inhalation or dermal absorption in the workplace. Nonoccupational exposure is uncommon but may occur via inhalation and oral routes from contamination of the environment (air and water) (NTP, 1998).

3. TOXICOKINETICS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

No information on THF absorption from the human gastrointestinal (GI) tract is available. However, blood and tissue concentration data from a toxicokinetic study in rats and mice conducted by DuPont Haskell Laboratory (1998) have demonstrated that THF is readily absorbed from the GI tract.

In this study, single gavage doses of approximately 50 or 500 mg/kg ^{14}C-THF dissolved in water were administrated to male and female F344 rats and B6C3F1 mice, and the level of THF-associated radioactivity in plasma was monitored for up to 168 hours. The mean values of selected toxicokinetic parameters for plasma identified in this study are presented in Table 3-1. In both rats and mice, radioactivity appeared in the plasma soon after the THF treatment, demonstrating the rapid absorption of THF from the GI tract. In rats, detectable levels of radioactivity were present in the plasma as early as 15 minutes after dosing (the earliest time point measured). Maximum plasma concentrations were reached after approximately 4 hours in the low-dose rats, and after 4–8 hours in the high-dose rats. In the low-dose group, the plasma concentration reached a maximum (C_{max}) of 19.8 μg THF equivalents/g in males at 4.0 hours and 13.8 μg THF equivalents/g in females at 3.0 hours. In the high-dose group, C_{max} was 71.6 μg THF equivalents/g plasma in males at 8.0 hours and 89.2 μg THF equivalents/g plasma in females at 3.2 hours (T_{max} in females was highly variable). Maximum plasma concentrations were not proportional to the administered dose, since C_{max} values differed by approximately fourfold for males and sevenfold for females between dose groups, while the administered dose differed by 10-fold. A similar evaluation of the plasma area under the curve (AUC) data revealed the same pattern of nonproportionality with dose. This phenomenon could reflect the saturability of absorption processes at high doses. Also, independent of absorption, dose-dependent changes in first-pass metabolism could possibly explain this result. Since GI tract absorption rates have not been measured directly, the data are not adequate to attribute the nonlinearity in maximum plasma concentrations or AUCs to absorption kinetics. As the values of many of the kinetic parameters are highly variable, the study authors (DuPont Haskell Laboratory, 1998) indicated that there were no gender differences for any of the kinetic parameters in the rat (statistical significance not reported by the study authors).

Table 3-1. Toxicokinetic parameters in rat and mouse

	50 mg/kg		500 mg/kg	
	Male	**Female**	**Male**	**Female**
Rat				
Actual dose (mg/kg)	40.3	45.9	428.7	478.3
T_{max} (hours)	4.0	3.0	8.0	3.2
C_{max} (µg equivalents/g)	19.8	13.8	71.6	89.2
$T_{1/2}$ (hours)	52.1	50.5	48.0	59.0
AUC (µg•hour/g)	535.8	319.6	2825.5	1998.0
Clearance (g/hour•kg)	75.2	143.6	151.7	239.4
Mouse				
Actual dose (mg/kg)	44.3	38.0	490.3	495.9
T_{max} (hours)	0.5	0.4	0.8	1.0
C_{max} (µg equivalents/g)	27.7	19.4	149.4	106.0
$T_{1/2}$ (hours)	56.9	51.4	57.3	98.5
AUC (µg•hour/g)	207.4	157.3	3237.9	1904.4
Clearance (g/hour•kg)	213.6	241.6	151.4	260.4

Source: Adapted from data in DuPont Haskell Laboratory (1998); data are expressed as mean values.

Similar to the observations in the rat, THF-associated radioactivity appeared rapidly in mouse plasma after gavage dosing. Fifteen minutes following the 50 mg/kg treatment, a mean value of 17.4 µg THF equivalents/g plasma was observed in females (no value was reported for males), and, following the 500 mg/kg treatment, mean values of 84.8 and 56.8 µg THF equivalents/g plasma were reported for males and females, respectively. In the 50 mg/kg dose group, plasma radioactivity reached the C_{max} of 27.7 and 19.4 µg THF equivalents/g at approximately 30 minutes after dosing in males and females, respectively. In the 500 mg/kg group, the plasma radioactivity reached C_{max} values of 149.4 and 106.0 µg THF equivalents/g at approximately 1 hour after dosing in males and females, respectively. No gender differences were observed for the mouse T_{max} values (statistical significance not reported by the study authors). The mouse T_{max} values were shorter than for the parallel dose-groups in rats, suggesting that the absorption of THF is more rapid in mice than in rats. As was observed in rats, the C_{max} values in mice were not proportional to the administered dose. However, evaluation of the plasma AUC data for mice suggested that the total absorbed dose was more than proportional to the administered doses; the AUC was 12-fold higher at the high dose in females and 16-fold higher than the AUC at the high dose in males as compared to the AUC in the corresponding low-dose groups. The lack of proportionality of the C_{max} and AUC is consistent with an effect of dose on absorption rate. However, effects of other kinetic parameters such as metabolism could explain these observations, and therefore the apparent nonlinearity in plasma kinetics cannot be attributed only to absorption.

The oral bioavailability of THF has not been assessed directly. However, measurement of THF-associated radioactivity in the excreta of the rats and mice in the toxicokinetics study by DuPont Haskell Laboratory (1998) suggests that most (if not all) of orally administered doses of

THF can be absorbed. In rats and mice, the total radioactivity recovered in urine, feces, expired air (CO_2 or volatile organics), tissues, cage wash, and residual feed was measured over a period of 168 hours after gavage dosing (Table 3-2). The total recovery of radioactivity (i.e., mass balance) was poor in both dose groups in rats and the high-dose group mice, which was attributed by the study authors to saturation in the CO_2 capture system at early time points after dosing and poor performance of the capture solvent for volatile organics. However, changes in the apparatus for collection of CO_2 and volatile organics employed for the low-dose mice yielded much better recovery of the administered radioactivity. Analysis of data from the low-dose group mice shows that little THF remains unabsorbed from the GI tract, since recovery of radioactivity in the feces did not account for more than 1.4% of the administered dose. The amount of THF-associated radioactivity recovered in the feces in these treatment groups was similar to the low-dose group mice, suggesting that THF is nearly completely absorbed following oral dosing of up to 500 mg/kg in rats and mice.

Table 3-2. Overall percent recovery of radioactivity following gavage

Sample[a]	50 mg/kg				500 mg/kg			
	Rat		Mouse		Rat		Mouse	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine	4.4	3.5	2.7	5.3	2.2	2.2	3.8	3.6
Feces	1.1	1.0	1.4	0.9	1.0	0.4	1.3	0.8
CO_2	47.8	47.5	58.2	74.6	21.9	18.8	51.1	36.2
Volatile organics	<LOD[b]	<LOD	17.8	24.5	<LOD	<LOD	0.3	0.2
Tissues	14.1	9.3	3.8	2.0	7.9	4.1	4.4	0.7
Cage wash & residual feed	<LOD	<LOD	1.3	1.2	<LOD	<LOD	1.1	1.9
Total	67.5	61.3	85.2	108.5	33.0	25.5	61.9	43.3

[a]This table contains data from only those individual rats that had all listed samples collected.
[b]LOD = Limit of detection.

Source: DuPont Haskell Laboratory (1998).

3.1.2. Respiratory Tract Absorption

The results from several human studies show that THF is readily absorbed from the respiratory tract.

A study of workers in a videotape manufacturing plant (Ong et al., 1991) suggested that THF is absorbed by the inhalation route. In a group of 58 workers, full shift personal sampling was conducted to estimate breathing zone concentrations of THF. THF concentrations in the blood, exhaled air, and urine of the workers were determined at the end of the final work shift of the workweek. Time-weighted average exposures ranged from 0.2–143.0 ppm (0.59–422 mg/m^3). The measured air concentrations correlated best with urinary THF levels (0.88),

followed by blood (0.68) and exhaled air (0.61). A limitation of the study was the inability to estimate the rate of THF absorption from the respiratory tract since the overall contribution of dermal exposure (described as extensive for some workers) and the systemic THF levels were not determined. It was also unclear whether dermal exposure might correlate with THF levels in breathing zone air. Another study of THF workers (Ong et al., 1991) reported that the degree of THF absorption from the respiratory tract is 70% under heavy workloads and 60% during normal breathing.

Kageyama (1988 [abstract available in English]) investigated the toxicokinetics of THF in human volunteers exposed by the inhalation route. In the first experiment, subjects (from 1 to 20 per group) were exposed for 6 minutes to THF concentrations of 108–395 ppm, and exhaled air was sampled. The authors calculated the THF uptake ratio based on the concentrations of THF in the inhaled air divided by the concentration of THF in the exhaled air. The average uptake ratio was 64.8% for males and 72.7% for females during normal breathing and 78.4% for males and 81.3% for females during deep breathing. No consistent concentration-related effects on uptake were apparent. These results suggested that as much as 81.3% of the THF was absorbed or retained in the lung under acute exposure conditions. In a second experiment, five male subjects were exposed for 3 hours to mean concentrations of 56 ppm THF, followed by a 1-hour recovery period and then a second 3-hour exposure. Exhaled air was monitored throughout the first 3-hour exposure period. The percentage of THF in expired air relative to inhaled air was reported as 40% during normal breathing and 27% during deep breathing. These results correspond to uptake ratios of 60% and 73%, respectively. The same results were observed for five male subjects exposed for a single 3-hour exposure period to a mean THF concentration of 193 ppm THF (experiment 3). The authors also exposed five male volunteers to approximately 200 ppm (207 ppm for first exposure and 178 ppm for second exposure) THF for sequential 3-hour exposure periods with a 1-hour recovery period in between (experiment 4). Blood samples were collected for several of the exposure protocols (experiments 2, 3, and 4). THF kinetics in the blood were highly variable among individuals. However, the appearance of THF in the blood demonstrates the systemic absorption of THF from the lungs in exposed humans.

Wagner (1974) also reported on the respiratory tract absorption of THF in four human volunteers. The volunteers were exposed to 100 ppm THF for 20 minutes. The absorption rate of THF was reported to be 60%. The author suggested that the reported absorption rate represented 80% of the steady-state absorption rate normally reached over a period of several hours. This value is similar to reports in other human volunteer studies (Teramoto et al., 1989; Kageyama, 1988).

Tissue distribution studies in animals also provide evidence for absorption of THF through the respiratory tract, since measurable levels of THF were found in a variety of tissues in rats exposed through the inhalation route (Elovaara et al., 1984; Kawata and Ito, 1984).

3.1.3. Dermal Absorption

Limited information is available on the dermal absorption of THF in either humans or animals. Systemic toxicity observed in acute dermal toxicity studies (Stasenkova and Kochetkova, 1963) showed that THF can be absorbed through the skin. Brooke et al. (1998) demonstrated that uptake of vapor of industrial solvents across the skin can also occur in humans, but the degree of dermal uptake appears to be negligible (compared to inhalation). Under the conditions of the study in which four volunteers, two with and two without masks, were exposed to 150 ppm THF vapor for 4 hours, dermal uptake of THF vapor (in volunteers with masks) was found to contribute around 1–2% of the body burden received following whole body (including inhalation) exposure (in volunteers without masks).

3.2. DISTRIBUTION

No tissue distribution studies have been conducted for humans exposed to THF by any route of exposure. However, Ong et al. (1991) reported that occupational exposures (potentially inhalation and dermal) to THF resulted in measurable blood and urine THF levels. Kageyama (1988) and Droz et al. (1999) reported measurable blood concentrations of THF in human volunteers exposed by the inhalation route. These results demonstrate the potential for wide tissue distribution of THF.

Tissue distribution of THF has been studied comprehensively in rats and mice following oral dosing (DuPont Haskell Laboratory, 1998). Single gavage doses of ^{14}C-THF at target concentrations of 50 or 500 mg/kg were administrated to male and female F344 rats or B6C3F1 mice, and radioactive residues were measured in the plasma, red blood cells (RBCs), skin, whole blood, bone marrow, brain, fat, heart, lungs, spleen, liver, kidney, GI tract and GI tract contents, ovaries, testes, adrenals, plasma, uterus, muscle, bone, and carcass.

For rats, plasma and RBCs were collected at multiple time points, and, at 168 hours after dosing, the animals were sacrificed and tissues were harvested for analysis of THF-associated radioactivity. The presence of radioactivity in plasma demonstrates that THF or its metabolites are available for systemic distribution. Comparison of kinetic data for plasma and RBCs provides information on partitioning of THF (or its metabolites) in the blood compartment. The C_{max} values for plasma were consistently higher than C_{max} values for RBCs, ranging from 2.7- to 4.8-fold among both dose groups in males and females. When the AUC data are compared for plasma versus RBCs, the opposite relationship is observed (i.e., AUC values are higher in RBCs than in plasma), consistent with the longer biological half-life in RBCs as compared to plasma (see Table 3-1). No data on protein binding in the plasma were available. These data suggest that THF-associated radioactivity partitions rapidly to the plasma, resulting in higher peak concentrations in the plasma than in RBCs.

Total recovery of the administered dose in tissues was minimal, ranging from 3.7 to 10.3% among the two dose groups in male and female rats. The highest percent recovery was in

the carcass, indicating that THF or its metabolites are widely distributed. Tissue-specific data on a concentration basis (µg equivalent THF/g tissue) are shown in Table 3-3. These data indicate that liver has the highest concentrations of radioactivity, followed by the fat and adrenal glands. Both male and female rats had similar patterns in the tissue distribution of THF-associated radioactivity at the two treatment doses, suggesting that at doses between 50 and 500 mg/kg no significant shift in relative target tissue doses would be expected.

Table 3-3. THF concentration in tissues at 168 hours following gavage

Tissue	Rat		Mouse		Rat		Mouse	
	Male	Female	Male	Female	Male	Female	Male	Female
	50 mg/kg				500 mg/kg			
	Tissue concentration (µg equivalent/g)							
Carcass	2.0	1.5	1.4	0.9	11.9	8.8	14.2	12.4
Skin	2.4	1.6	1.5	0.9	14.7	7.4	18.1	14.6
Whole blood	1.0	0.7	0.8	0.5	6.1	5.1	8.6	5.5
Bone marrow	3.7	2.9	1.1	2.4	17.0	9.4	0.2	9.9
Brain	2.1	1.3	1.4	1.0	8.3	7.7	12.3	10.0
Fat	4.1	3.0	3.1	2.2	31.3	14.0	35.7	20.5
RBCs (terminal)	1.8	1.2	1.2	0.9	8.5	8.1	12.8	8.8
Heart	1.7	1.4	1.0	0.8	10.1	7.8	11.6	9.0
Lungs	2.1	1.4	1.1	0.6	11.9	7.9	11.6	8.6
Spleen	2.2	1.1	1.0	0.7	9.5	6.6	12.9	9.1
Liver	15.4	11.9	1.4	0.9	60.5	38.3	17.9	12.9
Kidney	2.7	2.0	1.7	1.1	15.8	12.2	22.8	14.1
GI tract	1.8	1.0	0.9	0.6	8.4	6.0	11.3	8.0
GI contents	0.5	0.2	0.2	0.1	1.3	0.9	1.5	1.2
Ovaries	–	1.4	–	1.1	–	8.4	–	13.0
Testes	1.8	–	1.4	–	7.3	–	12.5	–
Adrenals	5.4	3.9	3.0	1.4	30.2	18.5	27.1	23.5
Plasma (terminal)	0.6	0.3	0.3	0.2	3.4	2.2	3.1	4.3
Uterus	–	1.1	–	0.8	–	7.8	–	8.5
Muscle	2.0	1.7	1.3	1.0	11.5	10.3	12.5	9.7
Bone	1.8	1.2	1.2	0.6	10.6	7.5	8.3	6.3

Source: Adapted from DuPont Haskell Laboratory (1998).

Similar to rats, THF-associated radioactivity appeared rapidly in the plasma of mice after oral exposure. Evaluation of kinetic parameters for blood compartments shows that peak concentrations are higher but total integrated doses (AUC) are lower in plasma versus RBCs. In mice the total percent of the administered dose recovered within 168 hours after oral dosing in these tissues ranged from 3.1 to 4.0%. The highest percent of the dose was recovered in the carcass, indicating that THF or its metabolites were widely distributed. Tissue-specific data on a concentration basis (µg equivalent THF/g tissue) at 168 hours are shown in Table 3-3. Tissue

distribution of THF-associated radioactivity was reported for male mice at multiple time points until terminal sacrifice at 168 hours after dosing. In the high dose males, peak concentrations were reached within 4 hours after dosing for all of the tissues studied, with peak concentrations notably higher in the adrenal glands, liver, and kidney. The rate of decrease in the levels of radioactivity was tissue dependent. Most notably, at longer time points, fat had higher levels of radioactivity than liver. At the low dose, the peak concentrations of radioactivity in the liver and kidney, but not adrenal glands, were higher than in other tissues. As in the high-dose group, the concentration of radioactivity in the fat of the low-dose group at 168 hours was higher than in other tissues measured.

Hara et al. (1987) investigated the distribution of THF by giving 300 and 700 mg/kg THF orally to male Wistar rats and rabbits (strain unspecified), respectively. Blood and tissue samples were collected for analysis of THF concentrations from groups of three rats at 10 and 30 minutes and 1, 2, 3, and 5 hours and from two rabbits at 7 or 8.5 hours after administration. No significant differences were found in the results between the two species. Ratios of tissue levels to blood levels were approximately 1.5–2.0 in adipose tissue and kidney and about 1.0 in the brain, liver, spleen, and muscle.

The distribution of THF has also been studied following inhalation exposures in animals. Elovaara et al. (1984) measured the distribution of THF into the brain and fat tissue of rats exposed to 0, 200, 1000, or 2000 ppm (0, 590, 2950, and 5900 mg/m^3) THF 6 hours/day, 5 days/week for 2–18 weeks. The exposed rats were sacrificed at 2, 8, 13, or 18 weeks, and THF concentrations were measured in the brain and perirenal fat. At all the time points, THF concentrations in the fat were consistently higher than in the brain by a factor of approximately two- to threefold. THF in both tissues increased as the THF concentration increased. As the treatment extended from 2 to 18 weeks, the THF concentrations in both tissues of the exposed rats gradually decreased. The authors suggested that the decrease in tissue levels with longer exposure duration was caused by the induction of the oxidative metabolism of THF, as evidenced by increases in liver and kidney 7-ethoxycoumarin O-deethylase activity (as a marker for metabolic enzyme activity) in THF-exposed animals beginning at two weeks (not duration dependent). However, the observed statistically significant increases in enzymatic activity appeared to reflect a decrease in the activity in control animals rather than an increase in activity in the treated animals. No changes in liver cytochrome P450 (CYP450) content were observed at the end of the study. Comparison of tissue levels of THF revealed, at the highest exposure concentration, that tissue levels were greater than the 10-fold difference in dose. This result is consistent with the greater partitioning of THF as the parent compound into fatty tissues as discussed above for the oral dosing study in mice.

Kawata and Ito (1984) compared the distribution of THF following several different inhalation exposure regimens. Male Wistar rats (5/control group and 25/experimental group) were exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure or for seven

daily 30-minute exposures. In addition, rats were exposed to 3000 ppm (8850 mg/m^3) THF vapor for 1 hour/day, 5 days/week for 12 weeks. THF concentration was determined in tissues immediately and 1, 3, 6, and 12 hours following the last exposure. Tissues evaluated in the study were the brain, thymus, lung, heart, liver, kidney, spleen, and blood. For the single exposure group, immediately after exposure the pattern of THF distribution in organs was: blood > brain = kidneys = heart > liver = spleen = thymus = lungs. Within 1 hour, differences among the tissue levels began to decrease, with only the lung levels being significantly lower and blood levels being significantly higher than the other tissues. No significant difference in THF levels was observed among the tissues within 3 hours postexposure. The authors (Kawata and Ito, 1984) suggested that lower levels of THF in the lung reflect elimination of unmetabolized THF from this organ. Although not discussed by the study authors, the lower levels of THF in the liver and kidney would be consistent with the metabolic capacity of these organs, since THF was measured as the parent compound in this study. Repeated exposure to 15,000 ppm resulted in a similar pattern of tissue level, except that immediately after exposure only the lung (significantly lower) and blood (significantly higher) levels were different from the other tissues.

In the rats that received 3000 ppm THF for 12 weeks, a different pattern of distribution was observed. Immediately after the last exposure, THF tissue levels were greatest in the thymus, followed by spleen > brain = heart > lung > blood > liver = kidney. The concentration of THF in thymus was significantly higher than THF concentration in other tissues and remained higher for up to 12 hours postexposure. Tissue levels of THF measured immediately after the last exposure for the 1-day and the 6- or 12-week 3000 ppm exposure regimens were compared. THF levels were proportionally higher with increasing duration of exposure from 1 day to 6 weeks, although for many tissues THF levels at 6 weeks were similar to those observed at 12 weeks. Daily tissue accumulation was most apparent for the thymus in which tissue concentrations were nearly twice as high as for the other tissues immediately after the last exposure at 12 weeks. Beginning at 6 weeks of exposure, THF concentrations were also notably higher in the spleen than in other tissues. Taken together, these data show that THF is taken up in the blood and is widely distributed following exposure by the inhalation route. Longer duration exposures may generate daily accumulation in some organs, although tissue levels decrease to background rapidly after cessation of exposure. THF distributed preferably to the thymus and spleen following subchronic exposures. The study authors suggested that higher THF concentrations in the thymus after longer-term exposures might reflect increased age-associated fattening of the thymus, corresponding to the normal age-related atrophy of this organ. However, the spleen was also noted as an organ with high tissue concentrations, suggesting to the study authors (Kawata and Ito, 1984) the possibility of THF distribution through the lymph system.

Pellizzari et al. (1982) reported the presence of THF in the milk from mothers who were living in one of four urban areas in the United States. THF was found in one of eight samples

that were analyzed. This study did not provide quantitative data on the concentrations of THF that were present or information on mothers' exposure.

No data on placental transfer of THF or fetal distribution is available in humans or in animal studies.

3.3. METABOLISM

Several lines of evidence suggest that THF undergoes oxidative metabolism by CYP450 enzymes.

In an in vitro experiment with hepatic microsomal preparations from rats, mice, or humans, the only metabolite that was identified was γ-hydroxybutyric acid (GHB) (DuPont Haskell Laboratory, 2000). The half-life for removal of THF in these reactions was 40 hours for rat microsomes, 28 hours for human microsomes, and 9 hours for mouse microsomes. The corresponding intrinsic clearance values were 30.6, 27.3, and 160.5 mL/min-kg, respectively. These data indicate that liver microsomes in mice have a greater capacity to metabolize THF than do human or rat microsomes. No data are available to confirm whether these relative rates of metabolism by microsomes are predictive of THF metabolism among species in vivo.

In vivo studies on THF metabolism indicated that CO_2 is the major terminal metabolite, as shown in Table 3-2 (DuPont Haskell Laboratory, 1998). In mice administered a single gavage dose of 50 mg/kg ^{14}C-THF, the percent of the radioactivity recovered as CO_2 was 58.2% in males and 74.6% in females. Volatile organics (possibly as unmetabolized THF) accounted for 17.8% of the administered dose in males and 24.5% of the administered dose in females. In mice administered a single dose of 500 mg/kg ^{14}C-THF, the percent of the administered dose recovered as CO_2 was 51.1% and 36.2%, for males and females, respectively. Rat metabolism studies also demonstrated that oxidative metabolism of THF to CO_2 is an important pathway. In rats given a single gavage dose of 50 mg/kg of ^{14}C-THF, 47.8% in males and 47.5% in females of ^{14}C-THF was recovered in the form of CO_2. In rats given 500 mg/kg of radiolabeled THF, these percentages were 21.9% in males and 18.8% in females.

In both sexes of mice and rats, metabolism of THF to CO_2 was greater at the low dose, suggesting that metabolism may be saturated at higher doses. Although the data suggest that there might be species differences in the contribution of CO_2 to THF metabolism, potential saturation of the CO_2 trap and therefore loss of CO_2 in the rat study make comparison of the rat and mice data unreliable.

Based on the in vitro metabolism data and the observed in vivo metabolism of THF to CO_2, the metabolic pathway presented in Figure 3-1 has been proposed (DuPont Haskell Laboratory, 2000). According to this pathway, THF undergoes oxidative metabolism to γ-butyrolactone (GBL), which is further metabolized to GHB. The metabolism of GBL and GHB has been studied extensively (National Sanitation Foundation International [NSF], 2003). GBL is rapidly metabolized in the blood and liver to GHB (Roth and Giarman, 1966). GHB can

be reduced to succinic semialdehyde, followed by oxidation to succinic acid. Succinic acid in its ionized form (succinate) is an intermediate in the citric acid cycle that undergoes a series of reactions, some of which ultimately lead to the release of CO_2. The CO_2 observed in the in vivo metabolism studies of THF may result from the oxidation of succinic semialdehyde.

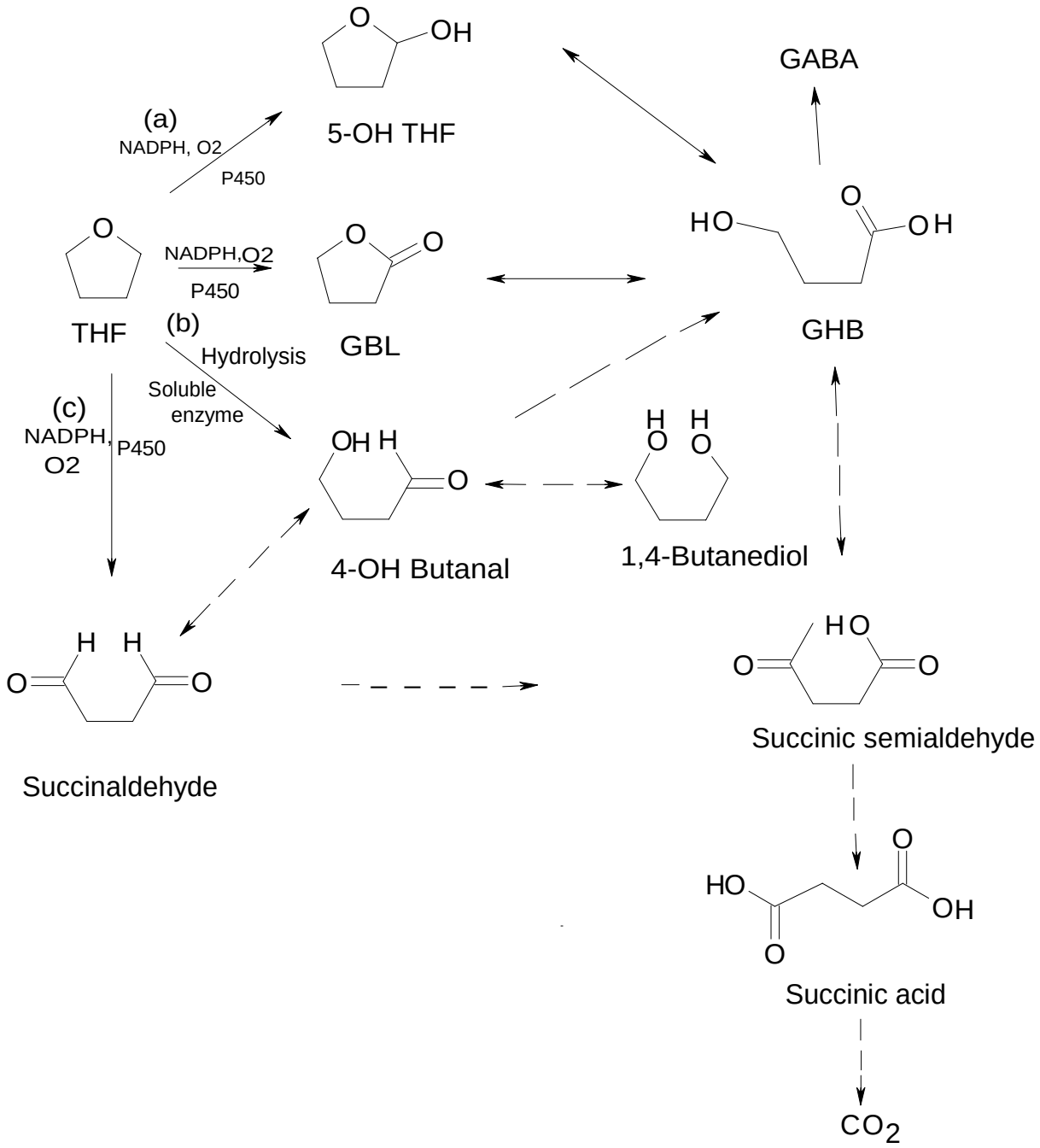


Figure 3-1. Possible metabolic pathways of THF.

Source: Adapted from DuPont Haskell Laboratory. (DuPont Haskell Laboratory, 2000).

The metabolism of THF to GBL appears to be supported by metabolic studies of p-dioxane, a structural analogue of THF. p-Dioxane-2-one, a lactone with a six-member ring analogous to GBL, has actually been identified as the major urinary metabolite of p-dioxane in rats (Woo et al., 1977). In addition, in vitro studies of structurally related compounds with a THF ring or similar ring structures indicate that there are several other possible pathways (see Figure 3-1) for the metabolism of THF to GHB, including (1) α-hydroxylation (by microsomal CYP450 enzymes) to 5-hydroxy-THF, which can be rapidly converted to GHB (Woo et al., 1977; Fujita and Suzuoki, 1973); (2) hydrolysis (by cytosolic soluble enzymes) to 4-hydroxybutanal, followed by immediate oxidation to GHB and GBL or reversibly reduced to 1,4-butanediol (El Sayed and Sadée, 1983; Roth and Giarman, 1968); and (3) oxidation to succinaldehyde (by microsomal CYP450 enzymes), followed by reversible reduction to 4-hydroxybutanal and oxidation to GBL or GHB in the presence of cytosolic soluble enzymes. The formation of GBL or GHB from succinaldehyde in soluble enzymes could also occur by oxidation to succinic semialdehyde, followed by reversible reduction (El Sayed and Sadée, 1983).

The implication of these metabolic intermediates to the overall toxicity of THF is unclear. Many of these intermediates (i.e., 2-hydroxy-THF, 4-hydroxybutanal, 1,4-butanediol, succinaldehyde) are expected to be unstable and rapidly undergo further metabolism to GHB. Studies in rats have shown that 1,4-butanediol is metabolized in the blood and brain to GHB and that GHB is the active intermediate responsible for the central nervous system (CNS) effects of 1,4-butanediol (Roth and Giarman, 1968). In fact, in vitro and in vivo studies have shown that GHB can be converted to the neurotransmitter γ-aminobutyric acid (GABA) (Vayer et al., 1985; DeFeudis and Collier, 1970), which provides a plausible mechanistic link between THF and its CNS effects. Appreciable amounts of radioactive-labeled GABA were detected in the brains of mice 60, 120, and 180 minutes after intraperitoneal (i.p.) injection of 1-^{14}C-GHB (DeFeudis and Collier, 1970). Increased tissue level of GABA and putrescine (the primary source of GABA in many tissues) has also been hypothesized to be a plausible basis for the THF-induced cell proliferation and carcinogenicity in the liver (see Section 4.6.3).

3.4. ELIMINATION

The available human data suggest that expiration is an important route of excretion for THF. In a human occupational study (Ong et al., 1991), workers exposed to THF by the inhalation and dermal routes excreted THF in exhaled air and in the urine. Kageyama (1988) measured exhaled air concentrations of THF in human volunteers exposed by the inhalation route. THF was present in the exhaled air for several hours after exposure to a concentration of 200 ppm, suggesting that THF is excreted in exhaled air. Droz et al. (1999) summarized the results from several additional human volunteer studies that support the conclusion that THF is rapidly excreted from the body via exhaled air and urine (personal communication and a non-

English conference proceeding). Exposure periods were for as long as 8 hours to concentrations as high as 200 ppm. In all cases, THF levels in breath, blood, or urine declined rapidly and reached background levels within a period of approximately 12 hours.

Oral dosing studies in animals provide further evidence for the important role that exhaled air plays as a route of excretion for THF. In rats exposed to an oral dose of 50 mg/kg THF, 47% of the oral dose was recovered in the expired air as CO_2, while only about 4% of the radioactivity was detected in the urine and 1% in the feces. In the mice exposed to the same dose of THF, 58–75% of the oral dose was recovered in expired air as CO_2 and 18–25% as volatile organic compounds (VOCs), while 3–5% of the radioactivity was detected in the urine and 1% in the feces. A similar pattern was observed in the animals exposed to the high dose of 500 mg/kg, but relatively less radioactivity, 19–22% as CO_2 in the rats and 36–51% as CO_2 in mice, was recovered in the expired air. Because of some technical difficulties in recovery of VOCs from the expired air, significant losses of trapped VOCs occurred in most of the measurements. Among all the data available for VOCs, the only reliable data were from the mice exposed to the low dose of THF. Nevertheless, all the available data indicate that expiration was the major route of excretion of absorbed THF, and CO_2 was the major final product. The study authors suggested that the VOCs in the exhaled air were likely to be parent THF. Urine and feces were relatively minor routes of THF excretion (DuPont Haskell Laboratory, 1998).

In the same study (DuPont Haskell Laboratory, 1998), the time course of THF in the plasma of exposed rats and mice was also studied. The results are summarized in Table 3-1. In the rats exposed to the low dose (50 mg/kg), the half-life ($T_{1/2}$) of the radioactivity in the plasma was 52 hours in the males and 51 hours in the females. Following exposure to the high dose (500 mg/kg) THF, the plasma $T_{1/2}$ was estimated to be 48 hours and 59 hours, respectively. In the mice exposed to the low dose, the plasma $T_{1/2}$ was 57 hours in the males and 51 hours in the females. Following exposure to the high dose (500 mg/kg) THF, the serum $T_{1/2}$ was 57 hours and 99 hours, respectively. Based on these data, there were no apparent differences in the plasma $T_{1/2}$ between rats and mice. At the 50 mg/kg dose level, male and female animals had a comparable $T_{1/2}$, while at 500 mg/kg THF the males had shorter plasma half-lives than the females. The half-lives reported in this study are not the biological half-lives of THF but only represent radioactivity measured in plasma and serum. The radioactivity present is likely derivatives of THF that are either covalently bound to cellular macromolecules or have been incorporated into the primary carbon pool. Available data indicate that the biological half-life of THF is about 5–7 hours. Hara et al. (1987) reported a half-life of 5.2 hours in rats, following oral administration of 300 mg/kg, and a half-life of 5.1 hours in rabbits at a dose of 700 mg/kg.

The AUCs for the THF-associated radioactivity in the plasma were estimated for the exposed rats and mice in the study conducted by the DuPont Haskell Laboratory (1998). In the rats exposed to 50 mg/kg THF, the plasma AUC in males and females was 536 and 320 μg THF equivalents-hour/g plasma, respectively. In rats exposed to 500 mg/kg THF, the plasma AUC in

males and females was 2826 and 1998 μg THF equivalents-hour/g plasma, respectively (see Table 3-1). At either the low or high doses, the AUC in the male rats was always higher than that in the female rats. A similar gender difference was observed in mice. In the 50 mg/kg dose group, the plasma AUC was 207 and 157 μg THF equivalents-hour/g plasma. The plasma AUC in males and females was 3238 and 1904 in the high-dose group (500 mg/kg), respectively. Based on these findings, the same oral dose of THF results in a higher internal dose of THF and/or its metabolites in male rats or mice than in females of the corresponding species. However, the toxicological implications of this result are difficult to interpret since the AUC reflects a combination of THF and its metabolites, while the toxic moiety has not been clearly identified. Nevertheless, in general, the greater AUC for males would be consistent with a greater degree of systemic dose in males versus females.

The AUC data from this study can be used to estimate the body clearance of THF. The clearance (Cl) was calculated based on the ratio of administered dose/AUC. All the relevant kinetic parameters and estimated Cl values are summarized in Table 3-1. In both rats and mice, females had a higher clearance rate than males. The more rapid clearance (i.e., due to lower AUC values) observed in females might reflect differences in excretion kinetics or alternatively might reflect differences in the degree of THF absorption, since the administered dose was used for this calculation rather than the absorbed dose. The clearance rates in the rats of the low-dose group were lower than the high-dose group, while there were no such differences in the mice.

Expiration as the major route of excretion of THF was also supported by Teramoto et al. (1989 [reported in a published abstract]). In rats administered an i.p. injection of 0.1 mL of THF, 40% of the dose could be recovered in the exhaled air as the parent compound. The biological half-life of THF in the exhaled air was 2 hours.

Kawata and Ito (1984) compared the blood and tissue distribution and elimination of THF, following several different inhalation exposure regimens. In male Wistar rats exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure, 70–80% of the THF was eliminated from the organs within 1 hour following exposure. After 1 hour, THF concentration decreased slowly and was almost completely eliminated by 12–13 hours following exposure. In animals that received seven exposures of 15,000 ppm, only 18–39% of THF was eliminated from the organs in 1 hour following exposure, indicating some saturability in the elimination kinetics for these organs at very high concentrations. In these animals, the rate of THF decrease was 31% at 3 hours following last exposure, 68% at 6 hours following last exposure; by 12 hours THF was almost completely eliminated. Similarly to the acute dosing studies, THF was nearly completely eliminated from blood and tissues within 12 hours after the last exposure in the 12 week exposure protocol. These data indicate that, for exposure concentrations as high as 15,000 ppm, THF is rapidly eliminated from blood and other tissues.

3.5. BIOACCUMULATION

Two toxicokinetic studies employed longer-term exposure regimens that provide information useful for assessing the potential for bioaccumulation of THF in tissues. Kawata and Ito (1984) measured tissue levels of THF immediately after the last exposure period following daily inhalation exposures to 3000 ppm THF for 1 day, 6 weeks, or 12 weeks. Daily levels increased in some tissues, particularly from 1 day to 6 weeks. In the thymus and spleen, tissue levels continued to increase through the 12-week exposure period. These data suggest some potential for tissue accumulation with repeated daily exposure. However, it is notable that even in animals exposed for 12 weeks, tissue levels declined rapidly after the end of the last exposure period (within hours). These data suggest that the rate of uptake of THF is more rapid than the rate of excretion. Therefore, during periods of continuous exposure, there is some potential for tissue levels of THF to accumulate. However, periods of intermittent exposure more typical of human exposure conditions would allow for clearance of the THF body burden and thus limit the potential bioaccumulation.

Elovaara et al. (1984) measured the distribution of THF into the brain and fat tissue of rats exposed to 0, 200, 1000, or 2000 ppm (0, 590, 2950, and 5900 mg/m^3) THF 6 hours/day, 5 days/week for 2–18 weeks. As the treatment extended from 2 to 18 weeks, the THF concentrations in both tissues of the exposed rats gradually decreased. The observed decline in brain and fat THF levels suggests that THF may not bioaccumulate in these tissues.

Evaluation of human volunteer studies to derive a physiologically based pharmacokinetic model for THF revealed rapid elimination of THF from the body (Droz et al., 1999). The resulting model predicted that no significant accumulation of THF would be expected over the workweek or across workweeks. THF elimination rates observed in inhalation (Elovaara et al., 1984; Kawata and Ito, 1984) and oral studies (DuPont Haskell Laboratory, 1998) in animals support this conclusion. Taken together, the data support the general conclusion that THF is not likely to bioaccumulate.

3.6. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

A human physiologically based toxicokinetic (PBTK) model has been developed by Droz et al. (1999) to estimate the THF concentrations in the blood, breath, and urine, following an inhalation exposure for the purpose of determining biological exposure indices in these media that would equate to an occupational exposure level of 200 ppm THF. The PBTK model was constructed with seven compartments: lungs, muscles and skin, fatty tissue, liver, kidneys, brain, and other tissues. Physiological parameters (tissue volumes, blood flow rates, etc.) were calculated from body weight and height and from physical workload by using formulas previously developed by the author (Droz et al., 1989). Blood-air and tissue-air partition coefficients were estimated from in vitro experiments. THF metabolism was assumed to follow first order kinetics. Urinary excretions were calculated assuming a urine flow of 1 mL/minute

and a creatinine excretion rate of 1.4 g/day. The model was validated by using four discrete sets of human exposure data from workers or human volunteer studies. The model provided an adequate fit to the data from three out of four sets of data. The reason for the lack of fit for one of these data sets was not determined. Based on the model predictions, repeated inhalation exposures to 200 ppm THF would yield end-of-the-work-shift levels of THF in biological samples of 5.1 ppm in breath, 57 μmol/L in the blood, and 100 μmol/L in the urine.

No PBTK models were identified for THF in animals.

3.7. SUMMARY

Overall, the available toxicokinetic data demonstrate that THF is readily absorbed through multiple routes, is systemically distributed, and is rapidly metabolized and excreted.

Inhalation studies in animals following subchronic exposures suggest that THF does not appear to bioaccumulate.

THF is readily absorbed from the respiratory tract, based on the observed rapid increase of THF in biological samples or calculated uptake rates in human studies (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988; Wagner, 1974). This conclusion is supported by the results of inhalation studies in animals (Elovaara et al., 1984; Kawata and Ito, 1984).

Although no human data are available to evaluate the rate or degree of absorption of THF following exposure through the oral route, oral dosing studies in rats and mice show that THF is readily absorbed from the GI tract (DuPont Haskell Laboratory, 1998). Peak plasma concentrations are reached over a period of hours, with apparent absorption rate in mice faster than in rats. Although the oral bioavailability has not been measured directly, the recovery of nearly all of the administered THF-associated radioactivity in exhaled air, urine, or tissues of mice (DuPont Haskell Laboratory, 1998) show that oral doses of THF are nearly completely absorbed.

No studies on dermal absorption were identified, but the observed systemic toxicity in a dermal toxicity study in mice and rabbits (Stasenkova and Kochetkova, 1963) demonstrated that THF can be absorbed through the skin.

Oral dosing studies with radiolabeled THF demonstrate wide tissue distribution of THF or its metabolites in mice and rats (DuPont Haskell Laboratory, 1998). THF-associated radioactivity partitioned rapidly in plasma and to a higher degree than in RBCs. No data on plasma protein binding were available. Total recovery of radioactivity in tissues represented only a small fraction of the administered dose, and the greatest proportion in tissues was in the carcass. On a tissue-concentration basis, the highest levels of radioactivity were found in the liver, kidney, and adrenal glands soon after exposure. At later time points, when excretion of THF from tissues was nearly complete, higher levels were observed in the fat. No dose-dependent pattern of tissue distribution was observed. THF is also widely distributed following exposure by the inhalation route, based on its presence in the blood and urine of exposed workers

(Ong et al., 1991) or human volunteers (Droz et al., 1999; Kageyama, 1988). Inhalation studies in animals support the results of the studies in humans. Elovaara et al. (1984) observed exposure concentration-dependent increases in brain and fat levels of THF in exposed rats. Kawata and Ito (1984) measured THF levels in multiple tissues in rats exposed acutely to high concentrations or during a subchronic exposure regimen. In the acute studies, tissues levels of THF measured immediately after the end of the exposure periods were notably higher in the blood and lower in the lung than in a variety of other tissues. A different pattern of tissue distribution was observed in rats exposed for 12 weeks, where the highest concentrations were in the thymus and the spleen. The authors suggested that there is a possibility for distribution of THF in the lymph system. In the subchronic exposure study, the lowest levels of THF were found in the liver and lung, which is consistent with the high metabolic capacity in the liver and exhalation of THF from the lung. Available data are not sufficient to evaluate the potential for distribution of THF to the fetus or to estimate lactational transfer.

Several lines of evidence suggest that THF undergoes oxidative metabolism by CYP450. First, a large percentage of orally administered THF is excreted as CO_2 (DuPont Haskell Laboratory, 1998). In both sexes of mice and rats, metabolism of THF to CO_2 was greater at 50 mg/kg than at 500 mg/kg, suggesting that metabolism was increasingly saturated at the high dose. Second, human, rat, and mouse microsomes, which are rich in CYP450 content, are able to metabolize THF (DuPont Haskell Laboratory, 2000). Third, THF is an inducer of CYP450 activity (Badische Anilin- und Sodafabrik [BASF], 2001a,b). Based on the identification of GHB and CO_2 as metabolites of THF (DuPont Haskell Laboratory, 2000), a metabolic pathway has been proposed in which THF is oxidatively metabolized to succinic acid. Succinic acid, in its ionized form (succinate), is an intermediate in the citric acid cycle that undergoes a series of reactions ultimately leading to the release of CO_2 from the parent molecule. In addition, in vitro studies of structurally related compounds with a THF ring or similar ring structures indicate that there are several other possible pathways for the metabolism of THF to GHB. Whether or not these metabolic intermediates contribute to the overall toxicity of THF is unclear. Many of these postulated intermediates (2-hydroxy-THF, 4-hydroxybutanal, 1,4-butanediol, succinaldehyde) are expected to be unstable and rapidly undergo further metabolism to GHB. GHB can be converted to the neurotransmitter GABA (Vayer et al., 1985; DeFeudis and Collier, 1970), which provides a plausible mechanistic link between THF and its CNS effects. Increased tissue level of GABA and putrescine (the primary source of GABA in many tissues) has also been hypothesized to be a plausible basis for the THF-induced cell proliferation and carcinogenicity in the liver (see Section 4.6.3).

The available human data suggest that THF is rapidly excreted. Excretion in exhaled air and urine were correlated with exposure concentration in an occupational study (Ong et al., 1991). Human volunteer studies demonstrate that THF is rapidly excreted in exhaled air and urine, with concentrations of THF in these tissues generally returning to background levels

within hours of the cessation of exposure (Droz et al., 1999; Kageyama, 1988). The rapid excretion of THF observed in human studies is supported by an inhalation study in rats (Kawata and Ito, 1984) in which tissue levels of THF decline rapidly during the postexposure period. THF is also rapidly cleared from the body following oral dosing, with exhaled air serving as the primary route of excretion (DuPont Haskell Laboratory, 1998). Analysis of the mass balance of radioactivity in the exhaled air, excreta, and tissues showed that nearly all of the administered dose was excreted in the exhaled air as CO_2 or volatile organics (possibly unmetabolized THF). The rate of excretion was rapid. The half-lives in the plasma were approximately 50 hours for most groups, although blood and tissue levels of radioactivity decreased rapidly, and tissue levels of radioactivity represented only a small percentage of the administered dose within 168 hours of exposure. Available data indicate that the biological half-life of THF is about 5–7 hours (Hara et al., 1987).

Inhalation studies in animals following subchronic exposures suggest that THF does not appear to be highly bioaccumulative. Elovaara et al. (1984) reported that brain and fat levels of THF decreased with increasing exposure duration. They attributed this result to the inducibility of enzymes responsible for the oxidative metabolism of THF. Kawata and Ito (1984) found higher tissue levels of THF immediately after the end of the last exposure period following a 12-week exposure regimen as opposed to tissue levels in rats exposed for 1 day or 6 weeks. However, even for the 12-week exposure protocol, tissue levels declined to background levels shortly after the exposure was ended. This result suggests that THF would only accumulate with uninterrupted periods of exposure.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

There are a number of human occupational exposure studies and case reports on humans exposed to THF. These human studies identify effects on the nervous system and liver. Most of these studies do not identify THF exposure levels. Also, all of the human studies report coexposures to other chemicals, including solvents that are neurotoxic.

Garnier et al. (1989) reported two cases of occupational exposure to THF. In both cases, the men (ages 35 and 55) worked as plumbers repairing pipes in confined spaces with a glue that contained THF. No information was given on possible exposure concentrations or the possibility of coexposure to other chemicals. Symptoms included nausea, headache, dizziness, chest pain, cough, dyspnea, and epigastric pain. In both men the clinical exam, blood count, and renal function were normal. The liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transferase (GGT) were elevated several times above the normal range. Clinical symptoms resolved in about 2 days and liver enzymes returned to normal within 2 weeks. The authors suggested that THF exposure may result in irritation, CNS effects, and transient liver toxicity in humans.

Emmett (1976) reported the case of a 41-year-old pipe fitter exposed for about 3 months to a mixture of THF and other solvents in a pipe cleaning solution and a pipe glue. Other solvents present in the solution included acetone and cyclohexanone. No information was provided on exposure concentrations. The only effects reported by the patient were a slight rhinorrhea during exposure and a gradual onset, over 10 weeks, of a constant unpleasant smell and loss of sense of smell. No other clinical signs were reported. A neurological exam, radiography of skull and sinuses, and hematological exam were all normal. Within 6 weeks after cessation of exposure, some sense of smell returned. However, by 7 months after the initial diagnosis, his sense of smell was still diminished.

Edling (1982) reported the occupational exposure of a shoemaker to a mixture of solvents that included THF, acetone, chloroform, and trichloroethylene. No information on exposure concentrations was provided. In addition, the patient had concurrent exposure to acetylsalicylic acid to treat lumbago. Clinical chemistry results revealed increased liver enzymes, including GGT and ALT. Liver biopsy showed centriacinar fatty change and siderosis.

Juntunen et al. (1984) reported cerebral convulsions in a patient following occupational exposure to both THF and enfluran anesthesia. The patient was a 45-year-old man who worked as a plumber, using a solvent containing THF to insulate the inside of a water piping system. For 2 weeks, the patient had been working with THF in enclosed spaces with no ventilation. No information was provided on the resulting exposure concentration. The patient reported that he

had felt unusually tired and had a headache in the week before he was admitted to the hospital for an appendectomy. On awakening from the enfluran anesthesia, the patient had several convulsions. In addition, liver enzymes were slightly elevated following the surgery. The authors concluded that THF exposure was the main contributing factor for the convulsions because the patient was exposed to high concentrations of THF for 2 weeks before the surgery. In addition he had never had epilepsy or neurological disease and his clinical status and computed tomography results were normal.

Albrecht et al. (1987) reported a case of autoimmune glomerulonephritis in a plumber working with pipe cement containing THF. The 28-year-old male plumber had been working with pipe cement for over 9 years. The initial symptom was gross hematuria. A needle biopsy of the kidney revealed segmental proliferative glomerulonephritis with immunoglobulin A deposits, capillary adhesions to the Bowman's capsule, and fibrin in the glomerular mesangial deposits. Industrial hygiene monitoring identified 15-minute exposures to THF, ranging from 389–757 ppm (1148–2233 mg/m^3) during periods that pipe cement was in use.

The National Institute for Occupational Safety and Health (NIOSH) (1991) investigated reports of adverse health effects at a plant that manufactured flexible hose. Environmental monitoring was conducted for respirable particulates, respirable silica, THF, total dust, metals, nitrosamines, and other organic compounds. Approximately 35–40 employees were interviewed by NIOSH investigators. In addition, the medical records of six employees who had sought medical attention for a work-related health problem and the death certificates of nine employees who were thought to have had work-related health problems were reviewed by NIOSH investigators. THF was detected in five air samples collected during a sealing operation. The concentrations ranged from 20–83 ppm (59–245 mg/m^3), but none of the sampling results were above the Occupational Safety and Health Administration standard of 200 ppm. However, the backup sections on the sampling apparatus also contained THF, indicating that breakthrough had occurred and suggesting that the THF exposure concentrations may have been higher. In addition to THF, other organic solvents detected in the air monitoring samples included acetone, toluene, methyl ethyl ketone, and 1,1,1-trichloroethane. The interviewed employees reported a variety of symptoms, including eye and respiratory tract irritation, headaches, lightheadedness, and drowsiness. The authors suggested that these symptoms may be related to solvent exposure but could not associate specific symptoms with individual chemicals.

Horiuchi et al. (1967 [only abstract available in English]) evaluated the health of workers employed in a vinyl chloride hose-manufacturing facility where THF was used as an adhesive. THF was detected in workplace air samples at concentrations as high as 1000 ppm (2950 mg/m^3). Workers who handled THF reported fatigue in the lower extremities. Clinical findings included decreased specific gravity of whole blood (more predominant in females), decreased white blood cell count, increased serum ALT activity, palpable liver, and hypotension.

Two human dermal THF exposure studies were identified. BASF (1938) did not observe contact dermatitis or sensitization in dermal tests of 1 in 96 human volunteers exposed to THF (exposure concentration not reported by study authors). Hofmann and Oettel (1954) reported that THF applied to the skin of six people produced irritation that was more severe when THF was allowed to evaporate. The authors concluded that THF itself was nonirritating, and the irritation was caused by impurities that remained after THF had evaporated away. No additional information was provided to evaluate the adequacy of this study.

The case studies described above suggest that THF exposures can induce a variety of noncancer effects, but no epidemiology studies are available that are sufficient to assess the carcinogenic or other long-term exposure-related health effects of THF in people. Capurro (1979) reported on cancer cases occurring in an area around a solvent recovery plant. According to the report, previous testing revealed a variety of solvents in the area (31 specific compounds were identified). A cohort of 117 residents having the highest levels of exposure was identified, and cancer mortality was tracked over a 6-year period. The authors reported a higher than expected number of cancer deaths in the exposed cohort (seven deaths observed versus one expected) but not in a second cohort living in the same county further from the solvent recovery plant. The complex array of exposures, which included well-documented genotoxic compounds in addition to THF, precludes the use of this study for assessing the carcinogenic potential of THF.

4.2. LESS THAN LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Less-than-Lifetime Studies

4.2.1.1. *Oral*

Komsta et al. (1988) reported the results of a short-term oral toxicity study of THF in rats. Sprague-Dawley rats (10/sex/group) were administered THF in drinking water at concentrations of 0, 1, 10, 100, or 1000 mg/L for 4 weeks. The equivalent doses estimated by the study authors based on measured water consumption and body weight were 0, 0.1, 0.8, 10.2, and 95.5 mg/kg-day. Clinical signs, body weight gain, and food and water consumption were evaluated weekly. Following the exposure period, the animals were sacrificed and examined at gross necropsy. Organ weights were obtained for brain, heart, liver, spleen, and kidney. Blood was collected for hematology and serum chemistry evaluation. A selection of tissues from the control and high-dose group was evaluated histopathologically.

There was no increase in mortality in any of the dose groups, and no clinical signs were observed in any of the treated animals. In addition, body weight gain and food and water consumption were not significantly different between treated and control animals. No changes in hematology or serum chemistry were observed in treated animals. Some sporadic observations of histopathological changes were observed in the thyroid, liver, and kidney; however, the

incidence for these findings was comparable in treated and control animals. Male rats in the high-dose group demonstrated a higher incidence of increased cytoplasmic homogeneity in liver compared with controls (3/10 and 7/10 for control and high-dose animals, respectively). Female rats showed an increased incidence of anisokaryosis (unequal size of cell nuclei) in the liver (0/10 and 7/10 for control and high-dose animals, respectively) and tubular cytoplasmic inclusions in the kidney (0/10 and 3/10 for control and high-dose animals, respectively). The authors did not conduct a statistical analysis of the incidence data. In addition, histopathology was not performed on the lower dose groups, so it is not possible to evaluate the dose-response relationship for these endpoints. Based on increased incidence of histopathology, the high dose of 95.5 mg/kg-day was an adverse effect level. However, since histopathology was not conducted for lower dose groups, the study is inadequate for determining a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL).

Pozdnyakova (1965) evaluated the effects of short-term exposure to THF in drinking water. White mice (number, sex, and strain not specified) received THF in the drinking water at concentrations of 40 and 100 mg/L for 45 days. Mice in the high dose group exhibited decreased body weight, paralysis of hind legs, leukocytosis, and decreased hemoglobin. No significant changes were observed in the low-dose group. No additional information was provided about the study.

In the same study report, Pozdnyakova (1965) exposed 20 rabbits (sex and strain not specified) and 50 white rats (sex and strain not specified) to THF in drinking water at doses of 10 and 20 mg/kg. The study was classified as being chronic in duration by the study authors; however, the actual duration of exposure was not specified. Rabbits in the high-dose group exhibited a change in cholinesterase activity, an increase in prothrombin time, and a low serum antibody titer compared with controls. Rats in the high-dose group showed a reduction in body weight and a change in serum albumin content. No additional information was provided in the study.

4.2.1.2. *Inhalation*

Horiguchi et al. (1984) evaluated the ability of THF to irritate the respiratory tract following short-term inhalation exposure to THF. Male Sprague-Dawley rats (three–six/group) were exposed to 0, 100, or 5000 ppm (0, 295, or 14,750 mg/m^3) THF vapor for up to 3 weeks. No information was provided on the duration of each exposure period or the number of days per week the animals were exposed, and therefore duration-adjusted exposure concentrations could not be calculated. A single animal was randomly selected from each exposure group 1 day, 1 week, and 3 weeks following the start of exposure. The animals were sacrificed the next day (24 hours later) and the respiratory tract mucous membrane was extracted and prepared for histological examination. No differences were observed between the tracheal mucosa of the treated groups and the controls following 1 day or 1 week of exposure. By 3 weeks of exposure,

the tracheal mucosa of animals in the high-concentration group exhibited disordered cilia and epithelial cells and darkening of cell bodies compared with control animals. Also by 3 weeks of exposure, the nasal mucosa of animals in the low-concentration group (100 ppm) exhibited the same type of changes described above for the tracheal mucosa nasal effects (e.g., disordered cilia and epithelial cells and darkening of cell bodies) without significant histopathological effects. The nasal mucosa of animals exposed to 5000 ppm for either 1 week or 3 weeks, however, demonstrated disruption of the epithelial architecture, congestion, and sloughing of ciliary and goblet cells, in addition to vacuolation and darkening of cell bodies. Based on these effects at the nasal mucosa, the LOAEL is determined to be 5,000 ppm and the NOAEL is 100 ppm.

Stasenkova and Kochetkova (1963) evaluated the short-term effects of THF inhalation in rats and mice (five/group; sex and strain not specified). The animals were exposed for 2-hour periods, twice a day, every day for 2 months. Air concentrations ranged from 6–8 mg/L (6000–8000 mg/m^3). However, THF vapor was generated by allowing it to evaporate from a filter paper, so constant air concentrations were not maintained for the duration of each exposure period. Animals were evaluated for clinical signs, mortality, and body weight. Endpoints evaluated included the threshold of neuromuscular irritability (method of measurement was not specified), arterial blood pressure, blood cell counts, liver function (measured by synthetic capacity), and kidney function (measured by albumin in urine). After 2 months, the animals were sacrificed and histopathological examination of major organs was conducted.

All animals developed symptoms of narcosis during the exposure; however, this effect was not observed during the periods between exposures. By day 40 of exposure, treated rats had reduced body weight compared with controls. At the end of the 2-month study period, mean body weight of treated rats was 30% less than controls. In addition, treated rats had a lower threshold of neuromuscular irritability than controls. No effects in rats were observed on blood pressure, blood cell count, or liver or kidney function. Histopathological lesions in the respiratory tract included catarrhal rhinitis, bronchitis, proliferative reaction in lungs, emphysema, and hypertrophy of muscle fibers in the walls of the bronchi. Histopathological lesions, including hypertrophy of muscle fibers and perivascular sclerosis, were observed in the heart, liver, and kidneys. Incidence data were not provided for any of these histopathological findings.

Treated mice initially developed symptoms of eye and respiratory tract irritation and had an increase in the threshold of neuromuscular irritability compared with controls. After 1 month of treatment, mortality in mice increased. The authors indicated that mice died of bronchial pneumonia. It was not clear if mortality in controls was increased and if the bronchial pneumonia was a cause of THF treatment or a bacterial infection in the mice. The mice still living at the end of the 2-month treatment period had a 15–20% decrease in body weight compared with controls. No information was provided on the results of other endpoints

evaluated in mice. Because of poor reporting of this study, no NOAEL/LOAEL can be determined.

Katahira et al. (1982) exposed groups of 10–12 male Sprague-Dawley rats by inhalation to 0, 100, 200, 1000, or 5000 ppm THF for 4 hours/day, 5 days/week over a 12-week period. Animals were evaluated for clinical signs, mortality, and body weight changes. Organ weights were measured for brain, heart, liver, lung, right kidney, and spleen. Standard hematology and clinical parameters were evaluated. Histopathological examination was performed on all tissues from the high-dose group and controls and on all gross lesions and target tissues from all dose groups. No significant differences were found among the groups exposed to 100 ppm as well as 200 ppm and the control group. The two high-dosed (1000 and 5000 ppm) groups showed an effect on the liver function. Specifically, significant increases in the activity of AST and ALT were observed. In the group exposed to 5000 ppm THF, the white blood cell count was significantly lower compared to controls, and marked local irritant symptoms and morphological damage of the respiratory mucous membrane were observed. The LOAEL of this study is 1000 ppm, and the NOAEL is 200 ppm.

In an NTP subchronic inhalation study (NTP, 1998; Chhabra et al., 1990), F344/N rats and B6C3F1 mice (10/sex/group) were exposed to target concentrations of 0, 66, 200, 600, 1800, or 5000 ppm THF vapor (0, 195, 590, 1770, 5310, or 14,750 mg/m^3) 6 hours/day, 5 days/week for 90 days. Animals were observed for morbidity and mortality, body weight, and clinical observations. Within 24 hours after last exposure, animals were euthanized, and blood and tissues were collected. All major tissues were fixed in formalin and processed. Histopathological examination was performed on all tissues from the high-dose group and controls and on all gross lesions and target tissues from all dose groups. Organ weights were measured for heart, liver, lung, right kidney, spleen, and thymus. Standard hematology and clinical parameters were evaluated in rats only.

In F344/N rats, body weight and survival were not affected by THF exposure. Clinical signs of ataxia, described as irregular movement with lack of coordination, were observed in both male and female rats at 5000 ppm but not at lower concentrations. In male and female rats at 5000 ppm, absolute and relative thymus (Table 4-1) and spleen weights were statistically significantly decreased. In female rats at 5000 ppm, absolute and relative liver weights were statistically significantly increased. Several hematological parameters in both male and female rats were significantly increased at 5000 ppm, including RBC counts, hemoglobin, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin (males only), segmented neutrophil count (males only), and platelet counts (females only). Female rats exposed to 5000 ppm had significantly increased levels of serum bile acids and significantly decreased blood urea nitrogen and creatinine. The only histopathological lesions observed in rats occurred in the forestomach at 5000 ppm. Acanthosis (increased thickness) was found in 5/10 males and 8/10 females, and suppurative inflammation of the forestomach was found in

2/10 males and 4/10 females. However, the authors concluded that forestomach lesions were the result of direct contact of THF ingested during the exposure period, rather than a systemic effect of inhaled THF. Based on observation of clinical signs and hematological effects, a concentration of 5000 ppm (14,750 mg/m^3) is determined to be a LOAEL and 1800 ppm (5310 mg/m^3) is a NOAEL.

Table 4-1. Rat absolute and relative thymus weights following subchronic treatment with THF

	Concentration (ppm)					
	0	**66**	**200**	**600**	**1800**	**5000**
Rats						
Males						
Absolute[a]	0.362 ± 0.015	0.353 ± 0.010	0.334 ± 0.009	0.345 ± 0.014	0.327 ± 0.015	0.279 ± 0.015[d]
Relative[b]	1.00 ± 0.03	1.00 ± 0.03	0.92 ± 0.015	0.95 ± 0.04	0.88 ± 0.03[c]	0.81 ± 0.04[d]
Females						
Absolute[a]	0.266 ± 0.010	0.262 ± 0.015	0.258 ± 0.010	0.248 ± 0.016	0.262 ± 0.010	0.212 ± 0.007[d]
Relative[b]	1.29 ± 0.04	1.26 ± 0.06	1.26 ± 0.04	1.17 ± 0.06	1.25 ± 0.04	0.99 ± 0.03[d]
Mice						
Males						
Absolute[a]	0.047 ± 0.003	0.045 ± 0.003	0.042 ± 0.002	0.039 ± 0.001[c]	0.036 ± 0.003[d]	0.027 ± 0.002[d]
Relative[b]	1.27 ± 0.06	1.23 ± 0.08	1.17 ± 0.05	1.08 ± 0.05[c]	0.99 ± 0.07[d]	0.81 ± 0.05[d]
Females						
Absolute[a]	0.051 ± 0.003	0.055 ± 0.003	0.056 ± 0.002	0.053 ± 0.002	0.052 ± 0.003	0.046 ± 0.003
Relative[b]	1.57 ± 0.09	1.71 ± 0.08	1.71 ± 0.10	1.64 ± 0.06	1.59 ± 0.11	1.36 ± 0.08

[a]Organ weights in grams.
[b]In mg organ weight per g body weight.
[c]$p \leq 0.05$.
[d]$p \leq 0.01$.

Source: Adapted from NTP (1998).

In B6C3F1 mice, body weights and survival were not affected by THF exposure (NTP, 1998). Male and female mice at both 1800 ppm and 5000 ppm showed clinical signs of CNS toxicity characterized as narcosis. At 5000 ppm, mice were in a stupor for 2 hours following the exposure period; at 1800 ppm mice were fully awake when chamber doors were opened following exposure. As with rats, CNS toxicity in mice decreased with increasing exposure duration. In male mice, relative liver weight was statistically significantly increased at concentrations of 200 ppm and both absolute and relative liver weights were increased at concentrations of 600 ppm. In addition, absolute and relative thymus weights were significantly decreased at concentrations of 600 ppm and absolute and relative spleen weights were significantly decreased at 5000 ppm only. In female mice, absolute liver weight was significantly increased at 5000 ppm. Absolute and relative weights of spleen, lung, and heart

were all significantly decreased at 5000 ppm. Histopathological lesions in mice were observed in liver, uterus, and adrenal gland. Liver centrilobular cytomegaly was observed in 7/10 male mice and 10/10 female mice at 5000 ppm (statistically significant) and 1/10 male mice at 1800 ppm. In addition, 10/10 female mice at 5000 ppm demonstrated uterine atrophy and degenerative changes of the adrenal cortex.

In assigning the adverse effect levels for this study, the clinical signs of narcosis observed at 1800 ppm in mice identify a clear adverse effect level. Liver and thymus weight changes were also considered as possible endpoints to serve as the critical effect. Statistically significant increases in liver weight were observed in males beginning at 200 ppm. However, the observed liver weight changes were not accompanied by histopathological changes until exposure concentrations reached 1800 ppm. Although the incidence of centrilobular cytomegaly (1/10) was not statistically significantly elevated at1800 ppm, this effect was considered to be treatment related based on the general correspondence between centrilobular cytomegaly and increased liver weight across the entire concentration range. The observed liver weight changes at 1800 ppm were considered to be a toxicologically relevant response. The thymus weight changes (see Table 4-1) observed at concentrations of 600 ppm and above in male mice were not considered to be a sufficient basis for assigning adverse effect levels. This judgment was made due to uncertainty regarding the toxicological significance of decreased thymus weight in the absence of functional immunotoxicity assays. In addition, the study authors suggested that the changes in thymus and spleen weights (in male and female mice, respectively) could be due to stress associated with THF administration. The LOAEL for this study is 1800 ppm (5310 mg/m^3) based on clinical signs of toxicity (narcosis) and supported by liver effects. The NOAEL is 600 ppm (1770 mg/m^3).

Horiguchi et al. (1984) evaluated the subchronic inhalation toxicity of THF in rats. Male Sprague-Dawley rats (11–12/group) were exposed to THF vapors 5 days/week, 4 hours/day for 12 weeks. Two experiments using different concentrations were conducted. THF concentrations for the first experiment were 0, 200, or 1000 ppm (0, 590, or 2950 mg/m^3) and for the second experiment were 0, 100, or 5000 ppm (0, 295, or 14,750 mg/m^3). Body weight and clinical signs of intoxication were observed daily during the exposure period. Rats were sacrificed on the second day following termination of exposure. Blood was drawn for hematological and serum chemistry evaluation. Major organs were weighed and evaluated histopathologically. Body weight in rats exposed to 5000 ppm was significantly lower than controls for the entire exposure period; no differences from controls were observed in the other treated groups. Animals in the 5000 ppm group displayed signs of local irritation and CNS effects, which were described by the study authors as similar to those observed for the acute study (Horiguchi et al., 1984). These local irritation and CNS effects were described as moderating with continued exposure. Serum AST was significantly increased above controls at concentrations of 200 ppm; however, the degree of increase in AST was minimal (the highest increase was 49% greater than controls at

1000 ppm) and was not considered sufficient to identify an adverse effect level. Cholinesterase and blood sugar were significantly increased at concentrations of 1000 ppm, and ALT, cholesterol, and bilirubin were increased only in the 5000 ppm group. ALT levels were doubled in the 5000 ppm concentration group as compared with controls. White blood cell count was significantly decreased compared with controls in the 5000 ppm group. Relative organ weights were significantly increased only in the 5000 ppm group, including brain, lung, liver, pancreas, spleen, and kidney weights. All histopathological findings were comparable between treated and control groups. Based on body weight and organ weight changes and on serum chemistry parameters, 5000 ppm (14,750 mg/m^3) is the study LOAEL and the NOAEL is 1000 ppm (2950 mg/m^3).

Kawata and Ito (1984) evaluated the health effects of THF following several different inhalation exposure regimens. Male Wistar rats (5/control group and 25/experimental group) were exposed to 15,000 ppm (44,250 mg/m^3) THF for a single 30-minute exposure or for seven 30-minute exposures. In addition, rats were exposed to 3000 ppm (8850 mg/m^3) THF vapor for 1 hour/day, 5 days/week for 12 weeks. Animals were observed for clinical signs and body weight. Blood was collected for serum chemistry analysis from animals exposed to 3000 ppm only. The following tissues were collected for histopathology: brain, thymus, lung, heart, liver, kidney, and spleen. Animals exposed to 15,000 ppm developed clinical signs of face-washing, shaking head, and rubbing face with paws. These behaviors were weaker and had shorter duration compared with those observed in rats that received repeated exposures (either seven 30-minute exposures to 15,000 or 3000 ppm for 12 weeks). In addition, rats receiving seven exposures to 15,000 ppm developed irritation of skin and mucous membranes as evidenced by severe salivation and nasal discharge. Rats exposed to 3000 ppm for 12 weeks also developed irritation symptoms that were milder than those observed at 15,000 ppm. No effects on body weight were observed after either single or multiple exposures to 15,000 ppm. However, by the fourth week of exposure, rats exposed to 3000 ppm had significantly reduced body weight compared with controls. Serum chemistry parameters were comparable between treated and control animals. No histopathological lesions were observed in either of the groups exposed to 15,000 ppm. In the animals exposed to 3000 ppm, histopathological lesions were observed in both lungs and kidney. Papillary hyperplasia and catarrhal degeneration were observed in lungs and bronchial epithelium. Protein casts and hyaline droplet degeneration were observed in the kidney tubule lumen epithelium in kidneys. Based on lung and kidney histopathological lesions, 3000 ppm (8850 mg/m^3) was determined to be a LOAEL. The study did not identify a subchronic NOAEL.

Stasenkova and Kochetkova (1963) also evaluated the effects of a 6-month inhalation exposure on rats. Male rats (20/group, strain not specified) were exposed to air concentrations of 1–2 mg/L (1000–2000 mg/m^3) 4 hours/day, 7 days/week for 6 months. Endpoints evaluated included clinical signs, body weight changes, blood cell count, blood pressure, and functional

condition of the neurovascular system, liver, and kidney. At the end of the 6-month treatment period, animals were sacrificed and histopathological examination of major organs was conducted. No effects were observed on behavior, body weight, liver function, kidney function, or neuromuscular irritability of treated rats compared with controls. Within 2–3 months of treatment, exposed rats developed increased numbers of leukocytes, which remained elevated compared with controls for the remainder of the experimental period. After 3–4 months, blood pressure in treated rats was reduced compared to controls, and this observation continued for the remainder of the treatment period. Histopathological lesions included mild hypertrophy in the muscle fibers of the bronchi walls and arteries of lungs and spleen.

BASF (1938) investigated the subchronic effects of THF exposure in dogs. Four dogs (strain and sex not specified) were exposed by inhalation to THF vapor at a concentration of 200 ppm (590 mg/m^3) 6 hours/day, 5 days/week for 9 weeks, followed by exposure to a concentration of 366 ppm (1080 mg/m^3) 6 hours/day, 5 days/week for 3 weeks. At the end of the 12 weeks, two of the four dogs were exposed on 2 successive days to a THF concentration of approximately 2100 ppm (5250 mg/m^3). Blood pressure was measured in dogs in the morning and afternoon for a 4-week control period and then before and after each daily exposure during the 12-week exposure period. Hematology, urinalysis, and limited pathological evaluations were also completed. In three of the 4 dogs, pulse pressure was depressed compared to the control period after three to four weeks of exposure to 200 ppm. In addition, increasing the THF concentration to 366 ppm resulted in a decrease in blood pressure compared to the control period in three of four dogs. In the two dogs exposed to 2100 ppm THF, a "sharp drop" in systolic, diastolic, and pulse pressure was reported by the study authors after the second day of exposure. No signs of narcosis or eye or respiratory tract irritation were observed in these two dogs. In one dog, hemoglobin decreased and white blood cells increased compared to the control levels. However, examination of the urine did not reveal any abnormality in kidney function. No gross or microscopic pathology was observed in the heart, lungs, spleen, pancreas, or kidneys of any of the dogs. Based on alterations in blood pressure, the study authors (BASF, 1938) reported a LOAEL of 200 ppm (590 mg/m^3). No NOAEL was identified by the study authors.

4.2.2. Chronic Studies and Cancer Bioassays

4.2.2.1. *Oral*

No chronic studies in animals by the oral route of exposure were identified.

4.2.2.2. *Inhalation*

NTP (1998) reported a study of the chronic toxicity and carcinogenicity of THF inhalation exposure in rats and mice. In the 2-year chronic study, groups of F344 rats and B6C3F1 mice (50/sex/group) were exposed to 0, 200, 600, or 1800 ppm (0, 590, 1770, or 5310 mg/m^3) THF for 6 hours/day, 5 days/week for 105 weeks.

Survival of treated rats was comparable to chamber controls at all exposure levels. Neither mean body weight differences nor clinical findings related to THF exposure were reported for either male or female rats. Pathology noted at sacrifice in male rats included marginal increases of renal tubular epithelial adenoma (at 600 and 1800 ppm) and two renal tubular epithelial carcinomas (at 1800 ppm), which, when combined, indicated a nonsignificant, treatment-related trend. The incidence of kidney tumor (adenoma and carcinoma combined) in male rats was 1/50 (2%), 1/50 (2%), 4/50 (8%), and 5/50 (10%) for doses of 0, 200, 600, and 1800 ppm, respectively. Although not statistically significant, the incidences of adenoma and carcinoma (combined) in the 600 and 1800 ppm males exceeded the historical range for chamber controls in the 2-year NTP (1998) inhalation studies. Table 4-2 summarizes the incidence of neoplastic and nonneoplastic changes in the kidney of male rats. No treatment-related changes in the incidence of neoplastic or nonneoplastic lesions in other tissues in the male or female rats were observed.

Table 4-2. Renal effects in male rats exposed to THF for 2 years

	Control	200 ppm	600 ppm	1800 ppm
Number of animals examined	50	50	50	50
Nephropathy, chronic	48[a] (3.0)[b]	50 (2.9)	50 (3.1)	50 (3.0)
Hyperplasia	7 (3.4)[b]	5 (3.6)	6 (2.5)	7 (3.3)
Mineralization	8 (16%)[c]	7 (14%)	2 (4%)	5 (10%)
Adenoma	1/50 (2%)	1/50 (2%)	4/50 (8%)	3/50 (6%)
Carcinoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adenoma or carcinoma[d]	1/50 (2%)	1/50 (2%)	4/50 (8%)	5/50 (10%)
Adjusted rate	8.3 %	16.7%	18.8%	38.3%
Terminal rate	1/12 (8%)	1/6 (17%)	0/5 (0%)	1/6 (17%)
First incidence (days)	733 (T)[e]	733 (T)	631	668
Logistic regression test[f]	$p = 0.037$	$p = 0.602$	$p = 0.159$	$p = 0.065$

[a]Number of animals with lesions.
[b]Average severity of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.
[c]Percent affected.
[d]Historical incidence for 2-year inhalation studies with chamber controls (6/652 [0.9 ± 1.3%]; historical control range 0–4%.
[e]T = terminal sacrifice.
[f]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 pair-wise comparison between the controls and the exposed group.

Sources: Adapted from Chhabra et al. (1998), NTP (1998).

No effects on survival or clinical observations were noted in female mice. Several statistically significant pathological changes were reported. These included concentration-related increases in hepatocellular adenoma and carcinoma that became significant in the 1800 ppm female treatment group. The incidence of hepatocellular adenoma in the control,

low-, mid-, and high-dose group was 12/50 (24%), 17/50 (34%), 18/50 (36%), and 31/48 (65%), respectively. For hepatocellular carcinoma, the respective incidences were: 6/50 (12%), 10/50 (20%), 10/50 (20%), and 16/48 (33%). The incidence of hepatocellular adenoma and carcinoma (combined) in the control, low-, mid-, and high-dose group was 17/50 (34%), 24/50 (48%), 26/50 (52%), and 41/48 (85%), respectively. A slight increase in liver necrosis was also observed in females exposed to 1800 ppm THF. Table 4-3 summarizes the incidence of neoplastic and nonneoplastic changes in the livers of female mice.

Table 4-3. Effects in female mice exposed to THF for 2 years

	Control	200 ppm	600 pm	1800 ppm
Number of animals examined	50	50	50	48
Eosinophilic focus	7[a]	9	7	11
Necrosis	3 (2.0)[b]	0	0	7 (1.9)
Adenoma	12/50 (24%)[c]	17/50 (34%)	18/50 (36%)	31/48 (65%)
Logistic regression test[d]	$p < 0.001$	$p = 0.249$	$p = 0.188$	$p < 0.001$
Adenoma, multiple	2/50 (4%)	3/50 (6%)	5/50 (10%)	12/48 (25%)
Carcinoma	6/50 (12%)	10/50 (20%)	10/50 (20%)	16/48 (33%)
Carcinoma, multiple	2/50 (4%)	4/50 (8%)	1/50 (2%)	6/48 (13%)
Adenoma or carcinoma[e]	17/50 (34%)	24/50 (48%)	26/50 (52%)	41/48 (85%)
Adjusted rate	46.3%	61.3%	69.1%	93.0%
Terminal rate	10/29 (34%)	18/33 (55%)	15/26 (58%)	29/32 (91%)
First incidence (days)	478	552	469	399
Logistic regression test	$p < 0.001$	$p = 0.188$	$p = 0.086$	$p < 0.001$

[a]Number of animals with lesion.
[b]Average severity of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.
[c]% affected.
[d]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 pair-wise comparison between the controls and that of the exposed group.
[e]Historical incidence: 200/937 (21.3% ± 11.9%); range, 3%–54%.

Sources: Adapted from Chhabra et al. (1998), NTP (1998).

In male mice, mean survival of the treated group was significantly less than chamber controls at 1800 ppm (average life span of 456 versus 689 days). As a result, the number of male mice available for evaluation of neoplastic changes at the termination of the study was small (12 animals compared to 32 animals in the control group). The only clinical observation was narcosis in male mice exposed to THF at 1800 ppm that lasted up to 1 hour following exposure. During periods of narcosis, the preputial fur was wet with urine, a condition that was thought to increase urogenital tract lesions and possibly lead to decreased survival. The lower survival rate and nonneoplastic pathology findings, including bone marrow and lymph node hyperplasia, hematopoetic proliferation of the spleen, and thymic atrophy, were considered by the study authors (NTP, 1998) to be secondary to the urogenital tract inflammation. Although the number

of male mice surviving to termination was small, statistical analyses for early mortality by NTP (1998) do not indicate that there is a treatment-related effect of THF on the incidence of liver tumor in male mice. Overall the only clear treatment-related nonneoplastic effect was clinical signs of toxicity (narcosis) in male mice at 1800 ppm (5310 mg/m^3).

Under the conditions of this 2-year bioassay, the NTP work group concluded that 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. There was *clear evidence* of carcinogenic activity of THF in female B6C3F1 mice due to increased incidences of hepatocellular adenomas and carcinomas.

4.3. REPRODUCTIVE/DEVELOPMENTAL TOXICITY STUDIES—ORAL AND INHALATION

4.3.1. Oral

BASF (1994) reported the results of a one-generation reproductive toxicity range-finding study in rats given THF in drinking water. Male and female Wistar rats (10/sex/dose) were given THF at concentrations of 0, 4000, 8000, or 12,000 ppm (equivalent to 0, 450, 775, or 1115 mg/kg-day) in the drinking water for 7 weeks prior to mating and throughout cohabitation, gestation, and lactation. Substance intake values estimated from measured water consumption and body weights are shown in Table 4-4. The F0 females were allowed to litter and rear pups (F1 generation) for 4 days postpartum, at which time the litters were culled to eight pups per litter (ideally four of each sex). Culled pups were sacrificed and examined for gross pathologic lesions, and the surviving F1 pups were sacrificed after weaning on day 21 postpartum. Clinical chemistry, hematology, and urinalysis parameters were measured in the F0 animals near the end of the study (approximately 12 weeks from initiating exposure), after which the F0 animals were sacrificed and assessed for gross pathology. Key treatment-related findings are also summarized in Table 4-4**.**

Food consumption was statistically significantly reduced in the high-dose F0 males and in the mid-dose F0 females. Water consumption was statistically significantly decreased in both sexes at the mid and high doses. No mortalities were recorded in either the F0 or F1 rats at any exposure concentration. THF exposure had no effect on any measured reproductive endpoint. However, relative kidney weights were statistically significantly increased in high-dose F0 males and in mid- and high-dose F0 females. In the F1 generation, numbers of pups, sex ratio, and viability/mortality were comparable to controls. Mean body weights and body weight gains of both male and female F1 pups were statistically significantly decreased in both the mid- and high-dose groups.

Table 4-4. Significant findings from one-generation reproductive toxicity study

Generation, sex		Concentration (ppm)			
	Parameter[a]	**0**	**4000**	**8000**	**12,000**
F0 Males	Substance intake[b]	0	444	795	1107
F0 Females	Substance intake:				
	Premating	0	467	798	1088
	Gestation	0	434	758	1139
	Lactation	0	714	1264	1847
	All periods	0	503	890	1240
F0 Males	Food consumption[c]	28.3 ± 1.81	28.1 ± 1.87	27.0 ± 1.57	25.9 ± 1.79[i]
F0 Females	Food consumption	19.9 ± 0.54	20.5 ± 0.72	18.8 ± 0.67	19.6 ± 0.62
F0 Males	Water consumption[d]	28.2 ± 1.80	26.8 ± 1.91	23.7 ± 1.60[i]	21.5 ± 1.94[i]
F0 Females	Water consumption	21.1 ± 0.92	19.8 ± 1.09	16.2 ± 0.73[i]	15.1 ± 0.87[i]
F0 Males	Body weight gain[e]	355.4 ±31.61	356.7 ± 32.09	342.0 ± 46.72	327.0 ±34.32
F0 Females	Body weight gain	104.6 ±14.62	115.7 ± 15.75	100.9 ± 9.94	104.4 ±12.42
F0 Males	Absolute kidney weight[f]	3.071 ± 0.178	3.032 ± 0.223	3.101 ± 0.289	3.141 ± 0.302
F0 Females	Absolute kidney weight	2.012 ± 0.157	2.115 ± 0.202	2.036 ± 0.12	2.153 ± 0.167
F0 Males	Relative kidney weight[g]	0.654 ± 0.047	0.647 ± 0.021	0.680 ± 0.036	0.705 ± 0.049[i]
F0 Females	Relative kidney weight	0.717 ± 0.034	0.735 ± 0.035	0.775 ± 0.04[i]	0.783 ± 0.048[i]
F1 Males	Pup body weight gain[h]	44.0 ± 3.16	42.4 ± 3.52	40.6 ± 3.18[i]	37.6 ± 5.33[i]
F1 Females	Pup body weight gain	42.7 ± 3.50	40.3 ± 2.60	38.0 ± 3.26[i]	36.2 ± 4.44[i]

[a]All values except for substance intake are shown as mean ± SD.
[b]Substance intake in mg/kg/day (mean). For males the intake during the premating period is shown. For females average intake are shown separately for premating, gestation, and lactation. For females the overall average intake was calculated as the time weighted average of the individual periods
[c]Food consumption over the premating period is shown as g/animal/day. No treatment-related effect on food consumption was reported for F0 females during gestation or lactation. For males the overall mean for the high concentration was not significantly different from controls, although food consumption rates for each of the weekly observations were significantly decreased.
[d]Water consumption over the premating period is shown as g/animal/day. For females, water consumption was also decreased during the gestation at the mid and high concentrations and during lactation at all THF concentrations. The overall mean for the mid and high concentration males and females over the premating period was not significantly different from controls, although each of the weekly observations were significantly decreased. Clinical chemistry did not show changes suggestive of severe dehydration.
[e]Body weight gain is shown in g. For males there was no significant change in BW gain over the entire study period. For females BW gain over the premating period is shown. No significant treatment related changes in BW gain were identified in females during gestation or lactation.
[f]Absolute kidney weight is shown in g. No treatment-related gross kidney pathology (histopathology was not performed) or changes in clinical chemistry, hematology, or urinalysis results were observed in males or females.
[g]Terminal kidney weight as % terminal body weight is shown.
[h]Pup body weight gain in g reported for days 4–21 postpartum.
[i]Statistically different ($p \leq 0.05$) from controls.

Source: BASF (1994).

Several treatment-related effects were evaluated as the basis for assigning effect levels for this study. Decreased water consumption was observed but did not induce clinical signs of dehydration and might simply reflect water palatability and therefore was not considered an

appropriate endpoint for assigning effect levels. Although a dose-dependent decrease in food consumption was observed, this effect was only observed at the high dose, and no corresponding statistically significant decrease in body weight gain was observed. Increases in relative kidney weight were statistically significant in F0 females exposed at the mid and high doses. Since the kidneys were weighed at the termination of the study, the appropriate dose metric for this endpoint is the time-weighted average substance intake for the entire study period. Therefore, the no-observed-effect level (NOEL) for increased relative kidney weight in F0 females is 503 mg/kg-day and the lowest-observed-effect level (LOEL) is 890 mg/kg-day. In the absence of changes in absolute kidney weight or clinical chemistry changes consistent with renal function impairment, the observed changes in relative kidney weight are of questionable biological significance. Thus, while an effect was seen at 890 mg/kg-day (i.e., at the LOEL), this dose is not the NOAEL for kidney changes. Statistically significant decreases in body weight were observed in the mid- and high-dose F0 and F1 females. Substance intake values for body weight effects on F1 pups are best represented by the time-weighted average substance intake for the gestation and lactation periods of F0 females. Therefore, the NOAEL for decreased body weight gain in F1 female pups is 546 mg/kg-day and the LOAEL is 960 mg/kg-day.

The results from this range-finding study were used in selection of exposure concentrations for a two-generation developmental and reproductive toxicity study of THF administered to rats in drinking water (Hellwig et al., 2002; BASF, 1996). Wistar rats (25/sex/group) received THF in their drinking water at concentrations of 0, 1000, 3000, or 9000 ppm (0, 104, 305, or 782 mg/kg-day) for 70 days prior to mating and throughout cohabitation, gestation, and lactation. Substance intake values estimated from measured water consumption and body weights are shown in Table 4-5. Before weaning, 25 F1 pups/sex/group were randomly selected to be the F1 parental animals. The remaining F1 pups were sacrificed. After the F1 generation pups were weaned, the F0 animals were sacrificed. The F1 animals were exposed continuously to THF at the same concentrations as their parents from weaning and throughout cohabitation, gestation, and lactation. After the F2 generation pups were weaned, the F1 generation parents were sacrificed. Endpoints evaluated in parental animals (both F0 and F1) include food and water consumption, body weight, mortality, and clinical signs. In addition, necropsy was performed on all parental animals at sacrifice, and organ weights were obtained for kidney, liver, testes, and epididymis. Histopathology was performed on all gross lesions, liver, kidney, reproductive organs, and gastrointestinal organs of sacrificed parental animals. Reproductive endpoints evaluated include mating index, fertility index, gestation index, and live

birth index. Litter/delivery endpoints for both F1 and F2 generations included total number of pups, number of live and stillborn pups, sex ratio, clinical signs, body weight, viability index, and lactation index. In addition, pups were evaluated for developmental stages (pinna unfolding, opening of auditory canal, opening of eyes) and behavioral tests (grip reflex, acoustic startle, pupil constriction). Culled pups, surplus pups, and all pups that died before weaning were assessed macroscopically, and, if abnormalities were found, the pups were evaluated by skeletal staining and histological processing of the head. Key treatment-related findings are summarized in Table 4-5.

Table 4-5. Significant findings from two-generation reproductive toxicity study

Generation, sex	Parameter[a]	Concentration (ppm)			
		0	1000	3000	9000
F0 Generation					
F0 Males	Substance intake[b]	0	91	268	714
F0 Females	Substance intake				
	Premating	0	104	301	742
	Gestation	0	104	288	790
	Lactation	0	166	478	1365
	All periods	0	112	322	835
F0 Males	Food consumption[c]	27.3 ± 1.35	27.2 ± 1.39	27.0 ± 1.48	26.5 ± 1.42
F0 Females	Food consumption				
	Premating	19.9 ± 0.61	20.0 ± 0.79	19.6 ± 0.73	18.3 ± 0.71*
	Gestation	25.0 ± 1.03	25.1 ± 1.28	24.4 ± 1.26	23.4 ± 1.38*
	Lactation	47.8 ± 12.34	47.4 ± 10.08	46.6 ± 10.17	46.0 ± 9.46*
F0 Males	Water consumption[d]	26.9 ± 1.35	25.8 ± 1.12	25.1 ± 1.03	22.0 ± 0.99*
F0 Females	Water consumption				
	Premating	20.6 ± 1.21	19.7 ± 1.15	19.1 ± 0.95*	15.1 ± 1.00*
	Gestation	32.2 ± 6.63	29.7 ± 6.10	27.9 ± 5.75*	24.3 ± 5.81*
	Lactation	57.3 ± 16.06	52.4 ± 12.70*	50.7 ± 12.05*	45.9 ± 11.27*
F0 Males	Body weight gain[e]	379.3 ± 53.52	378.3 ± 36.69	374.3 ± 40.73	364.2 ± 39.41
F0 Females	Body weight gain				
	Premating	138.3 ± 17.30	138.9 ± 16.31	141.7 ± 13.59	128.5 ± 14.25
	Gestation	129.7 ± 15.46	127.0 ± 14.01	124.7 ± 21.03	128.3 ± 15.82
	Lactation	9.7 ± 14.02	3.7 ± 12.10	9.9 ± 9.71	7.9 ± 9.53
F0 Males	Absolute kidney weight[f]	3.244 ± 0.301	3.203 ± 0.284	3.104 ± 0.272	3.438 ± 0.27[j]
F0 Females	Absolute kidney weight	2.092 ± 0.113	2.126 ± 0.142	2.159 ± 0.146	2.123 ± 0.133
F0 Males	Relative kidney weight	0.665 ± 0.052	0.662 ± 0.057	0.641 ± 0.059	0.719 ± 0.059[j]
F0 Females	Relative kidney weight	0.749 ± 0.039	0.774 ± 0.05	0.774 ± 0.054	0.785 ± 0.033[j]
F1 Male pups	Pup BW gain PND 4–21[h]	45.4 ± 3.04	46.3 ± 3.23	44.8 ± 3.63	41.7 ± 3.38[j]
	PND 1–4	3.0 ± 0.57	3.3 ± 0.96	2.7 ± 0.80	2.6 ± 0.53
	PND 4–7	6.1 ± 0.57	6.0 ± 0.71	5.8 ± 0.74	5.5 ± 0.75[j]
	PND 7–14	17.8 ± 1.15	17.5 ± 1.55	17.2 ± 1.43	15.7 ± 1.65[j]
	PND 14–21	21.4 ± 2.37	22.7 ± 1.80	21.9 ± 2.03	20.5 ± 1.84

Table 4-5. Significant findings from two-generation reproductive toxicity study

Generation, sex	Parameter[a]	Concentration (ppm)			
		0	1000	3000	9000
F1 Female pups	Pup BW gain PND 4–21	43.3 ± 2.72	44.0 ± 3.45	42.3 ± 2.61	40.1 ± 3.46[j]
	PND 1–4	2.8 ± 0.60	3.1 ± 0.85	2.7 ± 0.80	2.6 ± 0.51
	PND 4–7	5.9 ± 0.50	5.6 ± 1.10	5.5 ± 0.52	5.3 ± 0.65[j]
	PND 7–14	17.3 ± 1.47	17.4 ± 1.72	16.9 ± 1.66	15.6 ± 1.56[j]
	PND 14–21	20.1 ± 1.97	20.7 ± 1.86	19.9 ± 1.42	19.2 ± 1.84
F1 Generation					
F1 Males	Substance intake[b]	0	98	293	788
F1 Females	Substance intake				
	Premating	0	125	358	882
	Gestation	0	107	318	792
	Lactation	0	152	455	1165
	All periods	0	125	362	898
F1 Males	Food consumption[c]	28.0 ± 1.90	28.3 ± 1.77	28.1 ± 1.98	26.3 ± 1.99[j]
F1 Females	Food consumption				
	Premating	21.1 ± 0.50	21.4 ± 0.44	21.0 ± 0.44	20.9 ± 0.68
	Gestation	26.6 ± 1.53	26.7 ± 1.46	26.6 ± 1.33	26.0 ± 1.42
	Lactation	47.0 ± 13.63	44.8 ± 11.93	44.6 ± 12.69	40.5 ± 11.55[j]
F1 Males	Water consumption[d]	27.9 ± 2.07	29.2 ± 2.23	28.8 ± 2.65	24.2 ± 2.39[j]
F1 Females	Water consumption				
	Premating	23.5 ± 1.28	25.9 ± 1.63	24.0 ± 1.07	19.5 ± 0.89[j]
	Gestation	32.3 ± 7.74	33.8 ± 8.00	33.1 ± 6.54	27.7 ± 6.53[j]
	Lactation	57.0 ± 15.32	52.5 ± 10.82	52.1 ± 11.64	43.6 ± 10.93[j]
F1 Males	Body weight gain[e]	453.4 ± 40.49	456.6 ± 35.27	458.4 ± 53.88	426.1 ± 37.39
F1 Females	Body weight gain				
	Premating	198.0 ± 19.39	201.0 ± 22.92	204.5 ± 23.68	208.0 ± 24.49
	Gestation	127.1 ± 17.23	128.0 ± 14.22	125.0 ± 19.18	112.6 ± 17.79[j]
	Lactation	10.9 ± 13.44	4.6 ± 12.86	7.6 ± 10.99	9.4 ± 14.05
F1 Males	Absolute kidney weight[f]	3.233 ± 0.455	3.208 ± 0.192	3.201 ± 0.348	3.181 ± 0.338
F1 Females	Absolute kidney weight	2.347 ± 0.144	2.364 ± 0.201	2.365 ± 0.2	2.411 ± 0.153
F1 Males	Relative kidney weight[g]	0.62 ± 0.099	0.606 ± 0.041	0.608 ± 0.05	0.642 ± 0.058
F1 Females	Relative kidney weight	0.805 ± 0.048	0.8 ± 0.066	0.812 ± 0.043	0.826 ± 0.059
F2 Generation					
F2 Male pups	Pup BW gain PND 4–21[h]	42.6 ± 3.55	43.8 ± 4.67	41.5 ± 4.64	39.5 ± 3.13[j]
	PND 1–4	2.7 ± 0.85	3.0 ± 1.22	2.7 ± 1.00	3.0 ± 0.75
	PND 4–7	5.7 ± 0.95	5.8 ± 0.82	5.3 ± 1.15	5.0 ± 0.63[j]
	PND 7–14	17.4 ± 1.56	17.9 ± 1.98	17.0 ± 1.94	15.6 ± 1.67[j]
	PND 14–21	19.4 ± 2.23	20.2 ± 2.63	19.2 ± 2.07	18.9 ± 1.71
F2 Female pups	Pup BW gain PND 4–21	40.7 ± 3.67	41.2 ± 3.35	38.7 ± 4.67	38.1 ± 3.67
	PND 1–4	2.7 ± 0.71	2.7 ± 1.10	2.4 ± 1.11	2.9 ± 0.75
	PND 4–7	5.6 ± 0.75	5.2 ± 1.32	5.0 ± 1.12	5.0 ± 0.64
	PND 7–14	17.2 ± 1.50	17.1 ± 1.62	16.0 ± 2.41	15.4 ± 1.84[j]
	PND 14–21	17.9 ± 2.26	18.6 ± 1.83	17.8 ± 2.69	17.6 ± 2.15
F2 Male pups	Delayed eye opening[i]	89.9 ± 22.73	98.7 ± 4.13	94.0 ± 12.43	79.2 ± 31.18[j]

[a]All values except for substance intake are shown as mean ± SD. BW = body weight; PND = postnatal day.
[b]Substance intake in mg/kg-day (mean). For males, substance intake during the premating period is shown. For females, average intakes are shown separately for premating, gestation, and lactation, and the overall average intake was calculated as the time-weighted average of the individual periods.
[c]Food consumption over the premating period is shown as g/animal/day. No treatment-related effect on food consumption was reported for F0 or F1 males. For F0 females food consumption was decreased during premating, the first two weeks of gestation, and the second week of lactation for the high-concentration group, although decreases over these entire periods were not statistically significant. For F1 females, food consumption was decreased only during lactation in the high-concentration group.
[d]Water consumption is shown as g/animal/day. For F0 and F1 males, weekly water consumption was consistently significantly decreased at the high concentration, although the average over all weeks was not significantly different from controls. For F0 females, water consumption was consistently significantly decreased during gestation at the mid and high concentrations and during lactation at all THF concentrations. For F1 females, water consumption was consistently significantly decreased in the high concentration for all periods.
[e]Body weight gain is shown in g. For F0 and F1 males, body weight gain over the study period is shown, no significant change in body weight gain over the entire study period was reported for males, and terminal body weights did not differ among groups. For females, only body weight gain during gestation in F1 females was affected.
[f]Absolute kidney weight reported in grams. No treatment-related gross kidney histopathology was observed.
[g]Kidney weight as percent terminal body weight is shown.
[h]For F1 pups, body weight gain is presented in grams for selected postnatal periods. No significant difference from controls was observed for PND 1-4 or 14-21, although the average gain over days 4-21 was significant at the high dose in both males and females.
[i]Reported as % pups reaching criteria per litter
[j]Statistically different ($p \leq 0.05$) from controls.

Sources: Hellwig et al. (2002), BASF (1996).

In the F0 generation, food consumption of the high-dose group females was statistically significantly reduced during selected weekly measurements compared with controls during the premating period, gestation, and lactation. Water consumption for males in the high-dose group was statistically significantly decreased during the premating period, and for the mid- and high-dose group females it was statistically significantly decreased during the premating period, gestation, and lactation. In high-dose females, body weights were statistically significantly decreased compared with controls during selected periods during premating, gestation, and throughout lactation, but no significant change in body weight gain was observed. No clinical signs related to THF were observed in either F0 males or females at any dose. In F0 males, the mating index and fertility index were comparable among the controls and treated groups. Similarly, the mating and fertility indices for F0 females were comparable among control and treated groups. The mean duration of gestation was similar in all groups and the gestation index was 100% for all groups. Absolute kidney weight was increased in high-dose males, and relative kidney weight was significantly increased in both high-dose male and female F0 rats. No treatment-related gross lesions or microscopic findings were observed in either males or females. The total number of F1 pups delivered, the number of live and stillborn pups, and the sex ratio were comparable among the groups. In the low-dose group, nine F1 pups from a single litter died between days 1 and 10. Also, two dams in the mid-dose group cannibalized pups. Therefore, the lactation index for these dose groups is statistically significantly decreased

compared to controls. However, the authors concluded that this decrease is not related to administration of THF, because there was no dose-response relationship. The mean body weights and body weight gains of the F1 pups in the high-dose group were significantly decreased. The treated F1 pups did not demonstrate any clinical signs, changes in development stages, changes on behavioral tests, or findings on necropsy compared with controls.

Food consumption was significantly decreased in high-dose F1 male adult rats during the premating period and in high-dose F1 female rats during lactation. Water consumption was significantly decreased in high-dose F1 male rats during the premating period and in the high-dose F1 females during the premating period, gestation, and lactation. In high-dose F1 males, slight but significant decreases in body weight were observed throughout the study, but no effect on body weight gain was observed. No effects on body weight or body weight gain were observed in F1 female adults. No clinical signs related to THF were observed in either F1 males or females at any dose. In F1 males, the mating index and fertility index was comparable among the controls and treated groups. As well, the mating and fertility indexes for F1 females were comparable among control and treated groups. The mean duration of gestation was similar in all groups, and the gestation index was 100% for all groups. No treatment-related effects on organ weight, gross lesions, or microscopic findings were observed in either the male or female F1 adult rats at any exposure concentration.

The total number of F2 pups delivered was decreased in the high-dose group (not statistically significant). In addition, no decreases in the number of pups delivered were observed in the lower dose groups. The number of stillborn pups was statistically significantly increased in the two lower dose groups but not the high-dose group. Based on the lack of dose-response relationship, the authors concluded that all of these findings were spontaneous and not related to THF administration. In the low- and mid-dose groups, there was an increase in the number of pups cannibalized or dying before scheduled sacrifice. As a result, the viability index was statistically significantly decreased in the low-dose group; the viability indices for the mid- and high-dose groups were comparable to controls. Body weight gain was statistically significantly reduced in the high-dose group F2 pups but not in the lower dose groups. A significant number of F2 pups/litter in the high dose group had delayed opening of eyes and auditory canal compared with controls. Values for lactation index, sex ratio, clinical sign, behavioral tests, and necropsy findings were comparable between controls and treated animals.

In the high concentration groups, general toxicity was indicated by decreased food consumption, decreased body weight, and increased kidney weight in F0 adults and decreased food consumption and body weight gain in F1 adults. However, decreased adult body weights were only observed during selected periods during the study, were of minimal severity, and were not generally reflected by changes in body weight gain. Therefore, the adult body weight changes were not considered to be of sufficient magnitude to identify an adverse effect level.

No clinical signs (in the one- or two-generation studies) or clinical chemistry changes (only measured in the one-generation study) consistent with dehydration were observed, suggesting that the decrease in water consumption was not inducing dramatic changes in maternal health. However as noted above, data on the possible decrease in milk production as a result of decreased water consumption were not available from the available literature. One measure of effects of chemical treatment on pup development during the affected period is the lactation index (i.e., the number of live pups on postnatal day (PND) 21/number of live pups on PND 4 × 100), which was not affected by THF in this study. Since this response reflects changes in pup viability, it is only a gross measure and might not have captured more subtle effects of limited water intake decreases.

While the two-generation study shows a dose-response relationship between pup body weight gain and THF exposure, the contribution of other potential confounding factors, such as dam water intake (which may affect mother's milk production) and litter size (which may influence the milk availability to each pup), also need to be considered. To analyze the relationship between THF exposure and pup body weight gain and its relative importance compared with other possible confounding factors, multivariable regression analyses were conducted. The regression analyses included pup body weight gain during PNDs 7–14 as the dependent variable and four independent variables: average THF intake, maternal water intake duration lactation, number of pups in each litter (during the affected PND period), and a categorical variable for the dose group. Since the response data from F1 and F2 generation are independent, these data were analyzed separately. Preliminary regression analyses suggested that there was a high degree of colinearity among the independent variables as indicated by the high variance inflation factors and the dose group is the most significantly affected factor. Removal of this factor diminishes the colinearity in the regression. Therefore, in a second series of regression analyses, dose group was not included as an independent variable. The results from this regression analysis are summarized in Table 4-6.

Table 4-6. Correlations between water intake and decreased pup body weight gain

	Coefficient	*p* Value
F1 pup body weight gain (adjusted $r^2 = 0.36$)		
Average water intake	9.09×10^{-2}	<0.0001[a]
Average THF intake	-3.98×10^{-4}	0.1458
Number of pups	-4.23×10^{-1}	0.0335[a]
F2 pup body weight gain (adjusted $r^2 = 0.24$)		
Average water intake	5.90×10^{-2}	0.0015[a]
Average THF intake	-8.51×10^{-4}	0.0218[a]
Number of pups	-5.04×10^{-1}	0.0055[a]

Based on the results from multiple regression, the dependent variable (pup body weight gain) can be predicted from a linear combination of the independent variables of average water intake, THF intake, and number of pups in each litter. For F1 pups, there is no evidence to suggest a statistically significant correlation ($p = 0.1458$) between maternal THF intake and pup body weight gain when controlling the other confounding factors, such as maternal water intake and number of pups in each litter. However, the similar analysis for the F2 pup data indicates that there is a significant correlation ($p = 0.0218$) between pup body weight gain and maternal THF intake after controlling for the other confounding factors. Since THF intake contributes to the response, decreased pup body weight gain is an appropriate endpoint for deriving the RfD. This conclusion is consistent with the interpretation of the experimental results by the study authors who concluded that the high concentration effects reflect general toxicity of THF, while noting that decreased water (and food) intake could have contributed to the observed decrease in body weights.

Based on these analyses for parental (increased kidney weight and decreased body weight) and developmental effects (decreased pup body weight gain and delayed eye opening), the NOAEL is 3000 ppm and the LOAEL is 9000 ppm for this study. The best value to use for estimating the corresponding doses (mg/kg-day) differs for each generation, based on substance intake values over the relevant period of exposure. For parental effects, average substance intakes over the entire study period are appropriate for use in assigning effect levels. For developmental effects, the time-weighted average substance intake during the gestation and lactation period of the parent females was used to estimate the effective dose. Table 4-7 summarizes the corresponding effect level doses across all endpoints that showed a treatment-related effect. Based on these effect levels, the overall study NOEL for kidney effects is 268 mg/kg-day, with a corresponding study LOEL of 714 mg/kg-day and a NOAEL for kidney effects of 714 mg/kg-day. The overall NOAEL for developmental effects is 373 mg/kg-day, with a corresponding LOAEL of 941 mg/kg-day.

Table 4-7. Effect levels for the two-generation reproduction study

Effect	NO(A)EL (mg/kg-day)	LO(A)EL (mg/kg-day)
F0 Males—increased kidney weight[a]	268	714
F0 Females—decreased body weight, increased kidney weight[a]	322	835
F1 Pups—decreased body weight gain	364	1020
F1 Adult males—decreased body weight gain[a]	268	788
F1 Adult females—decreased body weight gain[a]	362	898
F2 Pups—decreased pup body weight gain and delayed eye opening	373	941

[a]The observed increases in kidney weight and decreases in adult body weight were of questionable biological significance; therefore, the values shown are the NOEL and LOEL and the highest dose would be a freestanding NOAEL.

Sources: Hellwig et al. (2002), BASF (1996).

4.3.2. Inhalation

Mast et al. (1992) assessed developmental toxicity of THF in mice and rats. Female CD-1 mice (10 virgin and 30 mated animals/group) were exposed to 0, 600, 1800, or 5000 ppm (0, 1770, 5310, or 14,750 mg/m^3) THF vapor for 6 hours/day, 7 days/week on gestation days 6–17. Female mice in the 5000 ppm group demonstrated a high toxicity, with >25% mortality observed after only 6 days of exposure. Consequently, mice in this group were removed from exposure at this time and placed in a chamber with fresh air until time of scheduled sacrifice. Developmental evaluations were conducted on pregnant mice euthanized on gestation day 18. Virgin females were sacrificed on the day after the last exposure. Developmental endpoints included gross maternal toxicity and number, position, and status of implantation sites. Live fetuses were weighed, sexed, and examined for gross defects. Half of the live fetuses and any fetus with gross defects were examined for visceral defects, and the heads were examined for soft-tissue craniofacial abnormalities. All fetal carcasses were examined for gross changes in cartilage and ossified bone. Maternal deaths occurred in the high-concentration group. Other statistically significant maternal effects that were observed at concentrations of 1800 ppm or higher included narcosis, decreased terminal body weight, reduced adjusted maternal weight gain (adjusted for uterine weight), and reduced gravid uterine weight. A reduction in the percent live pups/litter and delayed ossification of the sternum were observed at concentrations of 1800 ppm and higher. Surviving pregnant mice in the high concentration group had litters with a 95% resorption incidence; however, if the conceptus survived, development continued normally. There were no effects on the number of implantations, the fetal sex ratio, or the incidence of abnormalities in fetuses. Based on decreased gravid uterine weight in dams and reduced fetal survival, the LOAEL in mice is 1800 ppm (5310 mg/m^3) and the NOAEL in mice is 600 ppm (1770 mg/m^3).

Pregnant Sprague-Dawley rats (10 virgin and 30 mated animals/group) were exposed to 0, 600, 1800, or 5000 ppm (0, 1770, 5310, or 14,750 mg/m^3) THF vapor for 6 hours/day, 7 days/week on gestation days 6–19 (Mast et al., 1992). Developmental evaluations were conducted on pregnant rats euthanized on gestation day 20. Virgin females were sacrificed on the day after the last exposure. Developmental endpoints included gross maternal toxicity and the number, position, and status of implantation sites. Live fetuses were weighed, sexed, and examined for gross defects. Half of the live fetuses and any fetus with gross defects were examined for visceral defects, and the heads were examined for soft-tissue craniofacial abnormalities. All fetal carcasses were examined for cartilage and ossified bone. In dams, the cumulative body weights were significantly reduced in the high concentration group throughout the exposure period. In addition, nonsignificant reductions of gravid uterine weight and

extragestational weight gain (adjusted for uterine weight) were observed in the high concentration group. Fetal rat weights were significantly reduced at 5000 ppm. There were no effects on the number of implantations, fetal sex ratio, or incidence of fetal abnormalities. Based on decreased maternal and fetal weight, the LOAEL in rats is 5000 ppm (14,750 mg/m^3) and the NOAEL in rats is 1800 ppm (5310 mg/m^3).

DuPont Haskell Laboratory (1980) investigated the effects of inhaled THF on the developing fetus. The authors first performed a range-finding study in which Crl:CD® rats (7–14/group) were exposed to 0, 590, 1475, 7375, or 14,750 mg/m^3 6 hours/day on gestation days 6–15. In a follow-up study, Crl:CD® rats (29/group) were exposed to 0, 2950, or 14,750 mg/m^3 THF 6 hours/day on gestation days 6–15. Body weight, clinical signs, and feed consumption were observed in dams during the exposure period. Dams were sacrificed on gestation day 21 and were examined for gross pathologic changes, liver weight, and reproductive status. The number of corpora lutea, implantation sites, and live and dead fetuses were recorded. Live fetuses were weighed, sexed, and examined for external alterations. One-third of all fetuses and all stunted or malformed fetuses were examined for visceral alterations, and the heads were fixed for evaluation of eye malformations. Remaining fetuses were fixed and stained for examination of skeletal alterations. The same endpoints were examined in both parts of the study.

No mortality was observed in dams in either study. In both studies, dams in the high-concentration group demonstrated decreased response to noise stimulus, reduced muscle tone, and staggering gait that persisted for about 1 hour following each daily exposure period. In addition, dams in the lower concentration group (7375 mg/m^3 in the range-finding study and 2950 mg/m^3 in the main study) had a diminished response to noise stimulus. Food consumption in the main study high-concentration group was significantly reduced compared to controls. In both studies, dams in the high-concentration group had significantly reduced body weight gain compared to controls. The number of implants per dam and mean fetal body weight both were significantly decreased with increasing exposure (although no information is provided on which dose-level significance was first observed). In addition, fetuses in the high-concentration group exhibited a significantly decreased incidence of sternal ossification. Based on decreased fetal weight and skeletal alterations, the developmental LOAEL is 14,750 mg/m^3 and the NOAEL is 7375 mg/m^3. Based on clinical signs of sedation (diminished response to noise stimulus), the maternal LOAEL is 2950 mg/m^3 and the NOAEL is 1475 mg/m^3.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Studies

4.4.1.1. *Oral*

Hofmann and Oettel (1954) examined the effects of THF following oral exposure. Cats (13), rabbits (12), and rats (62, strain and sex not specified) received oral doses (route not specified) ranging from a single administration of 3 cm^3/kg (2670 mg/kg) to 25 administrations

of 1 cm^3/kg (890 mg/kg). The authors reported that no functional or histopathological damage to the liver was observed. Also, no changes were observed in urine analysis, serum urea content, or histopathology of the kidney.

Stasenkova and Kochetkova (1963) evaluated the acute toxicity of THF administered by gavage. White rats (10/group, sex and strain not specified) received THF doses of 1, 1.5, 2, 3, 4, or 5 g/kg by gavage as a solution in 2 mL of distilled water. The rats received a total of six doses. The rats were observed for clinical signs and mortality. Necropsy and histopathology of major organs was conducted in animals that died during the study exposure period. It does not appear that histopathology was performed on the animals that survived exposure. No mortality was observed at a dose of 2 g/kg. However, a dose of 3 g/kg resulted in 20% mortality, and doses of 4–5 g/kg resulted in 90–100% mortality, respectively. Clinical signs of sedation, including immobility, drowsiness, reduced response to external stimuli, and reduced respiratory rate, were observed after 3 to 9 minutes exposure. Mucous membranes appeared to have a cyanotic discoloration. Histopathological lesions were observed in the stomach, brain, liver, heart, spleen, and kidneys and included necrosis, edema, hemorrhage, and plethora (blood vessels or tissues filled completely with fluid).

Kimura et al. (1971) investigated the acute oral toxicity of THF in male Sprague Dawley rats (6–12/group). The median lethal dose (LD_{50}) values were estimated for four ages of rats: newborns (24–48 hours old), 14 days old, young adult (80–160 g), and older adult (300–470 g). Single doses of THF (doses unspecified) were administered by gavage; a microsyringe was used for the newborn animals. The oral LD_{50} values for THF were estimated as 2.3 mL/kg for 14-day-old rats, 3.6 mL/kg for young adult rats, and 3.2 mL/kg for older adult rats. The LD_{50} values for the young animals were not statistically different than the values for the older adult rats.

4.4.1.2. *Inhalation*

Stoughton and Robbins (1936) tested the effects of acute inhalation exposure to THF in both mice and dogs. Mice (10/group, strain and sex not specified) were exposed to THF concentrations of 0, 0.5, 1.0, 1.5, 2.2, or 3.0 mmol/L (0, 36,050, 72,100, 108,150, 158,620, or 216,300 mg/m^3) for a single 2-hour exposure. The parameters evaluated included the time required for onset of anesthesia and the time to respiratory failure or death. At the end of the 2-hour exposure, the animals still alive were observed until recovery or death. THF concentrations of 2.2 mmol/L were 100% fatal; at these concentrations, time to onset of anesthesia was 5–8 minutes and time to death was 30–51 minutes. The 1.0 mmol/L dose of THF resulted in 50% mortality, with time to anesthesia of 50 minutes and time to death of 109 minutes. No mortality was observed at a THF concentration of 0.5 mmol/L. Animals surviving at the end of the exposure period regained the ability to walk in 6–8 hours following exposure to THF. One dog (strain and sex not specified) was anesthetized with THF and

maintained for 1.5 hours at a THF atmospheric concentration of 5–6%. During this exposure, electroencephalogram (EEG), respiration, and blood pressure were measured. Two days following exposure, the dog was sacrificed and autopsied. Symptoms observed in the dog included increased saliva and mucus flow, decrease in blood pressure, stimulation of respiration, and prolonged sleep up to 6 to 8 hours after exposure stopped. No gross abnormalities were observed on autopsy.

Henderson and Smith (1936) exposed six rats (strain and sex not specified) to increasing concentrations of THF vapor for 1 hour. The exact concentrations of THF vapor used were not reported, but the authors note that anesthesia occurred at 6.47% THF. Two animals exposed to just the anesthetic concentration for one-half hour recovered within 2 minutes after exposure. Two rats that died within 24 hours of exposure had congested, mottled lungs. One rat that initially recovered but appeared ill 4 days later showed fatty changes in the liver.

Hofmann and Oettel (1954) examined the effects of acute inhalation exposure to THF in 18 cats, 20 rabbits, 52 rats, and 150 mice. The sex and strain of the animals were not specified. Animals were exposed to THF vapors at concentrations ranging from 3400–60,000 cm^3/m^3 (equivalent concentrations reported by the authors were 10,000–193,000 mg/m^3). Exposure regimens ranged from one 2-hour exposure to 30 6-hour exposures. No additional information was provided on exposure durations and concentrations. Therefore, it is not possible to estimate adjusted exposure concentrations. Liver function was assessed by using a bromosulfalein test (decreased clearance of bromosulfalein from the blood is indicative of liver dysfunction). Kidney function was also assessed by using urine analysis and serum urea content. Blood cell count was evaluated. Both the liver and kidney were evaluated histopathologically. The authors reported a slight, transient retention of bromosulfalein immediately following exposure to narcotic concentrations of THF.

LaBelle and Brieger (1955) evaluated the effects of acute THF inhalation exposure in rats and mice. Groups of eight male albino rats were exposed to a fixed concentration of THF for a single 4-hour exposure period. Those animals surviving were observed for 14 days. The range of concentrations tested was not specified. This procedure was repeated until the median lethal concentration (LC_{50}) could be determined. In addition, groups of white mice (six/group, sex not specified) were exposed continuously to saturated THF vapor (approximately 47,000 ppm or 138,650 mg/m^3), and survival time was recorded. For mice, the mean survival time following exposure to saturated vapor was 41 minutes. In rats, the LC_{50} reported by the authors was 18,000 ppm (53,100 mg/m^3). Narcosis was reported in rats prior to death.

Stasenkova and Kochetkova (1963) evaluated the effects of a single 2-hour inhalation exposure to THF in white mice and rats (10/group, sex and strain not specified). THF vapor was generated by allowing it to evaporate from a filter paper, so constant air concentrations were not maintained for the duration of the exposure period. For example, at the highest target concentration of 180 mg/L, air concentrations in the test chamber were reported as 140 mg/L

after 15 minutes and 65 mg/L after 2 hours. Based on the average of the measurements at 15 minutes and 2 hours, actual mean exposure concentrations were 0, 7, 13, 19, 27, 42, 73, 80, and 103 mg/L (0, 7000, 13,000, 19,000, 27,000, 42,000, 73,000, 80,000, and 103,000 mg/m^3). Animals were evaluated for clinical signs and mortality. Histopathological examination was conducted on animals that died. The authors did not indicate whether histopathological examinations were conducted on the animals that survived exposure. In mice, the average concentration of 19 mg/L resulted in 80% mortality, and 27 mg/L resulted in 100% mortality. Rats were less sensitive to THF. The average concentration of 42 mg/L resulted in 20% mortality, and 80 mg/L resulted in 100% mortality. The animals displayed symptoms of sedation and narcosis, including depressed activity, interrupted breathing, and reduced coordination of movement. In addition, mucus membranes were pale and bluish in color. Lesions observed in lungs and bronchi include plethora, edema, perivascular hemorrhage, and catarrhal condition of the mucus membrane. Histopathological lesions were also observed in brain, liver, kidney, and spleen, including plethora, edema, and dystrophic changes.

DuPont Haskell Laboratory (1979) conducted an acute inhalation study of THF in order to determine the highest concentration of THF that would not produce narcosis in rats. ChR-CD rats (six/sex/group) were exposed to THF concentrations, ranging from 3010–20,500 ppm (8880–60,475 mg/m^3) for a single 6-hour exposure period. Following exposure, all rats were weighed daily and clinical signs were observed for 14 days. The authors determined that the nonnarcotic concentration in male rats was 5380 ppm (15,871 mg/m^3) and in female rats was 5700 ppm (16,815 mg/m^3). During the exposure period, both male and female rats demonstrated clinical signs of pawing and scratching and decreased or no response to sound at all concentrations. Male rats also exhibited signs of rapid respiration, and females showed signs of paralysis. Based on clinical signs of CNS toxicity, the lowest exposure concentration of 8880 mg/m^3 is the study LOAEL.

Ohashi et al. (1983) evaluated the effects of acute inhalation exposure to THF on the upper respiratory tract (nasal mucosa) of rabbits. Adult rabbits (sex and number not specified) were exposed to THF concentrations of 100, 250, 1000, 2000, 6000, or 12,000 ppm (295, 738, 2950, 5900, 17,770, or 35,400 mg/m^3) for a single 4-hour exposure period. The rabbits were sacrificed by air embolization, and their nasal mucus membranes were obtained at 0, 20, 40, 60, 120, or 180 minutes following exposure. The membranes were evaluated for ciliary beating frequency and examined by scanning electron microscopy. No other organs or systems were evaluated. THF caused a dose-related decrease in ciliary beating frequency. Concentrations of 250 ppm caused about a 50% decrease in beat frequency; although, at these concentrations, beat frequency returned to normal within 3 hours following exposure. Concentrations of 1000 ppm almost completely eliminated ciliary beating, and at these concentrations beat activity did not return to normal within 3 hours. THF concentrations of 250 ppm resulted in the appearance of sporadic compound cilia but no other morphological changes. Concentrations of 1000, 2000,

and 6000 ppm resulted in the increased incidence of compound cilia and the vacuolation of epithelial cells, indicating moderate degeneration. At 12,000 ppm THF, observations included many large compound cilia, vacuolation, cytoplasmic protuberances, and sloughing of the epithelial cells, indicating severe degeneration. Based on significant morphological changes to nasal epithelial cells, 1000 ppm (2950 mg/m^3) is the LOAEL and 250 ppm (738 mg/m^3) is the NOAEL.

Horiguchi et al. (1984) evaluated the acute toxicity of THF following inhalation exposure in rats. Sprague-Dawley rats (six males/group) received a single 3-hour exposure to THF at concentrations of 200, 1000, 5000, 10,000, 15,000, 25,000, or 30,000 ppm (590, 2950, 14,750, 29,500, 44,250, 73,750, or 88,500 mg/m^3). The animals were observed for clinical signs of toxicity, abnormal behavior, and mortality for 72 hours following exposure. The LC_{50} value was estimated to be 21,000 ppm (61,950 mg/m^3) by using a probit method. Animals in the 200 ppm group displayed signs of head shaking and face washing, as well as patches of mild irritation on nose, ears, and eyelids, and sleep. Symptoms of irritation increased with increasing THF concentrations. At 5000 ppm, animals displayed intense salivation, tearing, and bleeding from the nose. In addition, animals developed clonic muscle spasms, had altered respiratory patterns, and became comatose about 1 hour following the start of exposure. All animals in the 25,000 ppm group died within 72 hours following exposure. No information was provided regarding the observations in other dose groups. Based on clinical signs of irritation and neurotoxicity, the concentration of 5000 ppm (14,750 mg/m^3) appears to be the LOAEL in this study.

Ikeoka et al. (1988) investigated the effects of acute inhalation exposure to THF on the lower respiratory tract (tracheal mucosa) of rabbits as a follow-up to the earlier study by Ohashi et al. (1983). Adult rabbits (sex and number not specified) were exposed to THF at concentrations of 100, 250, 1000, 2000, 6000, or 12,000 ppm (295, 738, 2950, 5900, 17,770, or 35,400 mg/m^3) for a single 4-hour exposure period. The authors did not state if a control group was also included. The rabbits were sacrificed by air embolization, and their tracheal mucosa membranes were obtained at 0, 20, 40, 60, 120, or 180 minutes following exposure. The membranes were evaluated for ciliary beating frequency and examined by scanning electron microscopy. No other organs or systems were evaluated. THF caused a dose-related decrease in ciliary beating frequency. Concentrations of 250 ppm caused about a 50% decrease in beat frequency that returned to normal within 3 hours following exposure. Concentrations of 1000 ppm almost completely eliminated ciliary beating, and at these concentrations beat activity did not return to normal within 3 hours. Compound cilia, ballooning, and vacuolation of tracheal epithelial cells were observed in the high-concentration group. However, the areas of severe degeneration observed in the nasal epithelium following the same exposure protocol were not observed in the trachea in the current study. The effects on the tracheal morphology were mild compared with those observed in nasal epithelium by Ohashi et al. (1983). Based on tracheal

histopathology, 12,000 ppm (35,400 mg/m^3) is the LOAEL and 6000 ppm (17,770 mg/m^3) is the NOAEL.

4.4.1.3. *Dermal*

Stasenkova and Kochetkova (1963) evaluated the effects of THF administered to the skin of white mice (20, strain and sex not specified) and rabbits (number, sex, and strain not specified). Pure THF (1 mL) was applied to the skin of rabbits. THF caused reddening of the skin, which subsequently thickened and sloughed off. Pure THF applied to the eyes of rabbits caused edema of the eyelid, vasodilation, and corneal opacity. The tails of mice were immersed in pure THF for 2 hours. This treatment resulted in mortality, symptoms typical of THF poisoning, and plethora and hemorrhage of internal organs.

4.4.2. Neurotoxicity Studies

DuPont Haskell Laboratory (1996a [published in the peer-reviewed literature as Malley et al., 2001]) investigated the neurotoxicity of acute inhalation exposure to THF in rats. Crl:CD BR rats (12/sex/group) were exposed to THF vapor at concentrations of 0, 500, 2500, or 5000 ppm (0, 1475, 7375, or 14,750 mg/m^3) for a single 6-hour exposure (designated as test day 1). The animals were then observed for 2 weeks following exposure. Clinical signs, body weight, and food consumption were evaluated weekly. The response to an alerting stimulus was determined as a group for each exposure concentration, prior to the start of exposure and approximately 2 and 4 hours after initiation of exposure. All rats were evaluated for neurobehavioral effects. Motor activity assessments and functional observational battery (FOB) assessments were conducted before exposure and on test days 2, 8, and 15. For the motor activity assessments, animals were individually tested in an automated activity monitor that measured both duration of continuous movements and number of movements. The FOB assessment consisted of a series of quantified behavioral evaluations conducted in a sequence that proceeded from the least interactive to the most interactive. During the FOB assessment, each rat was evaluated in three environments: inside the home cage, on removal from the home cage while being handled, and in a standard open field arena.

Exposure to 2500 ppm THF appeared to have an effect on response to alerting stimulus in rats. Six of 24 rats in the 2500 ppm group had a diminished response after 2 hours of exposure, and all 24 rats in this group had diminished response after 4 hours of exposure. Half the rats in the 5000 ppm group had diminished response after 2 hours of exposure, and all of the rats had either no response or diminished response to stimulus after 4 hours of exposure. Other signs of sedation in the high concentration group included a significant increase in the incidence of lethargy and abnormal gait in both male and female rats at 5000 ppm. Male rats in the 5000 ppm group had significantly decreased body weight gain and food consumption in the interval between test day 1 and 2, although these values were comparable to controls for the remainder of

the observation period. Several parameters in the FOB were affected in the 5000 ppm groups immediately following the exposure period only, including the righting reflex in males and females, palpebral closure in females, and ease of handling in females. The effects on FOB parameters were not observed during test days 2, 8, or 15, suggesting that the sedative effects of THF were short-lived. The LOAEL for this study is 2500 ppm (7375 mg/m^3), based on observations of sedative effects, and the NOAEL for this study is 500 ppm (1475 mg/m^3).

DuPont Haskell Laboratory (1996b [published in the peer-reviewed literature as Malley et al., 2001]) investigated neurotoxicity following subchronic inhalation exposure to THF in rats. Crl:CD BR rats (12–18/sex/group) were exposed to THF vapor at concentrations of 0, 500, 1500, or 3000 ppm (0, 1475, 4425, or 8850 mg/m^3) for 6 hours/day, 5 days/week over a 13- to 14-week exposure period. Clinical signs, body weight, and food consumption were evaluated weekly. Prior to the start of exposure and approximately 2, 4, and 6 hours after initiation of exposure, the response to an alerting stimulus was determined for the rats as a group for each exposure concentration. All rats were evaluated for neurobehavioral effects. Motor activity assessments and FOB assessments were conducted before the first exposure and at 4, 8, and 13 weeks. For the motor activity assessments, animals were individually tested in an automated activity monitor that measured both duration of continuous movements and number of movements. The FOB assessment consisted of a series of quantified behavioral evaluations conducted in a sequence that proceeded from the least interactive to the most interactive. During the FOB assessment, each rat was evaluated in three environments: inside the home cage, after removal from the home cage while being handled, and in a standard open field arena. Rats (six/sex/group) were sacrificed after 13 weeks of exposure, and tissue from the nervous system and muscle was assessed histopathologically.

The only effects observed in this study appeared to be related to the acute sedative effects of THF. A diminished response to alerting stimulus during exposure was observed in male and female rats in the 1500 and 3000 ppm exposure groups. In the 3000 ppm group, diminished response was observed consistently, beginning on the second day of exposure, in the 1500 ppm group. Diminished response was observed sporadically from days 16–49 of exposure and observed consistently on the remaining exposure days. Compound-related clinical signs, including stained nose and stained/wet perineum, were also observed in male and female rats in the 1500 ppm and 3000 ppm groups. These signs were not observed on Mondays prior to the start of exposure for the week nor on the days of the motor activity and FOB assessment. Therefore, these signs were considered to be transient. No effects were observed on body weight, body weight gain, food consumption, motor activity, any of the parameters in the FOB, or neuropathology in either male or female rats at any concentration. Based on clinical signs of sedation during exposure to THF, 1500 ppm (4425 mg/m^3) is the study LOAEL, and the NOAEL for this study is 500 ppm (1475 mg/m^3). However, the authors suggested that these effects were transient.

Kawata et al. (1986 [available as an abstract only]) measured the effect of inhalation exposure to THF on catecholamine levels in rat brain. Rats (number, strain, and sex not specified) were allowed to inhale THF vapor at a concentration of 15,000 ppm (44,250 mg/m^3) for either a single 30-minute exposure or a series of seven 30-minute exposures. Another group of rats was exposed to 3000 ppm (8850 mg/m^3) THF vapor for 3 hours/day, 5 days/week for 8 weeks. Noradrenaline and dopamine contents in the brain were measured following the end of exposure. In rats that received a single 30-minute exposure to THF, noradrenaline content in the cerebellum was immediately significantly decreased; dopamine content in the cerebrum significantly increased 48 hours after exposure. In rats receiving seven 30-minute exposures, the dopamine content of the cerebrum decreased significantly 48 hours after the last exposure period. In rats exposed to 3000 ppm for 8 weeks, the noradrenaline and dopamine contents in the cerebrum were higher than the control values (not significant) immediately after exposure but then decreased 48 hours later. Due to the limited extent of the available data in the abstract, no critical effect levels were assigned for this study.

Marcus et al. (1976) evaluated the neuropharmacological effects of THF administered by i.p. injection. Male Sprague-Dawley rats (number/group not specified) were implanted with electrodes to facilitate continuous EEG recordings. THF was administered by i.p. injection at doses of 15 and 21 mmol/kg (1156 and 1619 mg/kg). After a 2-minute latency period, 21 mmol/kg THF induced high amplitude slow wave activity in the EEG, which lasted 2 minutes. The EEG pattern then changed to spiking and electrical silence, which lasted for 20 minutes. The altered EEG pattern was accompanied by loss of the righting reflex. A dose of 15 mmol/kg induced a desynchronization of the EEG activity without loss of the righting reflex.

In an in vitro study, THF caused a decrease in adenosine triphosphatase (ATPase) activity and membrane fluidity in a dose-dependent manner in an assay using rat brain synaptosomes (Edelfors and Ravn-Jonsen, 1992).

4.4.3. Mode-of-Action Studies

THF was evaluated in a series of short-term in vitro assays to assess its potential for cytotoxicity (Curvall et al., 1984): inhibition of cell growth of ascites sarcoma BP 8 cells grown as stationary suspension cultures, inhibition of oxidative metabolism in isolated brown fat cells, plasma membrane damage (leakage of a cytoplasmic nucleotide marker from prelabeled cells), and ciliotoxicity as measured by time to ciliostasis in cultures of trachea from unborn chickens. To facilitate comparison of multiple chemicals, the results from each individual assay were expressed as a percentage of control responses and then these percentages were converted to a 10-point scale where 0 corresponded to 0–9%. The response observed in each of the individual assays of THF was less than 10%. THF was scored 0 for each of the individual assays and for its mean cytotoxicity activity. In contrast, several chemicals, mostly alkylphenols, were highly active in the test systems, having activity of 7 in each of the test systems. In a related study, a

5 mM concentration of THF took >60 minutes to cause ciliostasis in an in vitro assay in embryonic chicken trachea, whereas highly cytotoxic compounds caused ciliostasis in <5 minutes (Pettersson et al., 1982). Therefore, the results of these studies suggest that THF is not cytotoxic.

The cytotoxicity of THF was evaluated in an in vitro assay of protein content in cell cultures (Dierickx, 1989). Human hepatoma, HepG2 cells were maintained in culture in 24-well tissue culture test plates. THF and other test compounds were dissolved directly in culture medium at five different concentrations (not specified) and incubated with test cells for 24 hours. The cells were lysed and protein content measured. The relative toxicity of THF and the other test compounds was determined by estimating the concentration in mM required to induce a 50% reduction of cell protein content (PI_{50}). Very toxic compounds, such as heavy metals and surfactants, consistently had PI_{50} values of less than 1 mM. In contrast, the PI_{50} for THF was 372. The results of this study suggest that THF is not cytotoxic.

The cytotoxicity of 168 chemicals, including THF, was characterized as part of a validation of the BALB/c-3T3 cell transformation assay (Matthews et al., 1993). The LC_{50} for THF was 90.3 mM. The authors noted that in the analysis of the entire data set of 168 chemicals, in vitro cytotoxicity did not correlate to in vivo carcinogenic activity. THF was considered by the authors as noncytotoxic (defined as having an LC_{50} ranging from 5 to 100 mM).

The nervous system may be a primary target of THF toxicity. Metabolism data (see Section 3.3) suggests that the nervous system effects of THF may be due to its metabolites, GBL and GHB. The data for these compounds is only briefly summarized in this document to facilitate an evaluation of THF toxicity data, but a more detailed review is available (NSF, 2003).

GBL is used as a chemical intermediate and a solvent in many consumer product applications and as a food additive (Adams et al., 1998). GBL and GHB have also been used in medical applications as anesthetics (historical use) and in the medical treatment of addiction and fibromyalgia. GHB has become recognized as a drug of abuse, stemming from its sedative and hypnotic effects.

The systemic toxicity of GBL has been investigated in a full 2-year bioassay in rats and mice that employed gavage dosing (NTP, 1992). The most sensitive effect observed in these studies was clinical signs of CNS toxicity (lethargy) with a NOAEL of 112 mg/kg-day in rats. The only other treatment-related effect observed in rats and mice was for decreased body weight. Thymic depletion was observed in high dose male mice. The authors attributed this reduction to stress induced by fighting in high-dose males. The increased incidence of thymic depletion was similar in both the low- and high-dose males. The relevance of the thymus effects remains uncertain.

In other studies, no prenatal developmental effects were observed in rats at doses up to 500 mg/kg-day (Kronevi et al., 1988), while decreased testicular weight was reported in a short-term reproductive study (Debeljuk et al., 1983) with a LOAEL of 667 mg/kg-day.

The oral toxicity data for GHB are primarily from clinical studies in human subjects or from case reports of oral poisonings. Transient dizziness and a sense of dullness in 50% of human subjects following a single oral dose of 12.5 mg/kg were observed by Ferrara et al. (1999). Standardized measure of psychomotor performance was not affected at this dose (Ferrara et al., 1999). Metcalf et al. (1966) reported the effects of single oral doses of 35–63 mg/kg GHB in human volunteers. All participants reported drowsiness during the experiment, and some participants receiving doses over 50 mg/kg were rendered unconscious. Medical anesthetic doses of GHB are typically in the range of 60 mg/kg (Miotto et al., 2001; Vickers, 1969; Root, 1965).

BASF (1998) reevaluated kidney tissues from male rats and liver tissues from female mice from the NTP (1998) study to examine the relationship between cell proliferation responses and increase in tumors observed in these tissues following THF administration. Histopathological examination and evaluation of cell proliferation as measured by proliferating cell nuclear antigen (PCNA) staining were conducted using tissue samples from the 0, 200, 600, and 1800 ppm (0, 590, 1770, and 5310 mg/m^3) exposure groups (10/group) from the NTP (1998) subchronic (13 weeks) study. For the male rat kidneys, tissues from the cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla were evaluated separately. For the female mouse liver, no zonal subdivision was made.

The histopathology examination revealed increased incidence of moderate grade hyaline droplet accumulation in the male rat kidney tissues of the high-concentration group as compared to controls, but these changes were not accompanied by evidence of cell degeneration. No other differences between controls and exposure groups were noted. No increase in cell proliferation was found in any of the kidney compartments or in evaluation of all compartments combined. Cell proliferation index was statistically significantly decreased in individual kidney compartments, although these changes did not show a concentration-dependent pattern. For the female mouse liver tissues, no treatment-related histopathology was observed. The cell proliferation index was increased by approximately 39% in tissues from the high-concentration mice compared with controls. However, this result was not statistically significant and was noted as being predominately based on the results from 2 of 10 animals. Furthermore, a significant decrease in proliferation index was observed in the mid-concentration group, but no clear concentration-response pattern was observed. Based on these results, the study authors concluded that the examination of the tissues from the 13-week NTP (1998) study revealed no clear increase in cell proliferation that can be correlated to a tumorigenic mechanism.

BASF (Gamer et al., 2002; BASF, 2001a) evaluated a series of endpoints in male F344 rats (6/group plus 5/group at the control and high concentrations for enzyme assays) and female B6C3F1 mice (10/group plus 5 in the control and high concentrations for enzyme assays) in tissues for which THF-treated animals developed tumors. Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed

by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed nose only to average THF concentrations of 0, 598, 1811, or 5382 mg/m^3 (0, 199, 604, or 1794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Concentrations adjusted for continuous exposure were 0, 107, 323, or 961 mg/m^3. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the kidney (rat) and liver and uterus (mouse). Additional evaluations in these same organs included measurements of cell proliferation (S-phase response by 5-bromo-2-deoxyuridine [BrdU] staining) and TUNEL (terminal deoxynucleotidyl transferase deoxyuridine triphosphate [dUTP] nick-end-labeling staining) apoptosis assay. For the male rat kidneys, immunohistochemical detection of α_{2u}-globulin was also performed. Five animals from the control and high-concentration groups that were exposed for 5 consecutive days were also harvested for measurement of CYP450 content and for CYP450 isozyme activity as measured by ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-dependentylase (PROD) activity.

No clinical effects, body weight changes, kidney weight changes, or gross pathology related to THF exposures were reported for male rats. In the low-concentration group, no gross or histopathological effects were observed. Additional mode-of-action studies in the kidneys to evaluate cell proliferation, apoptosis, and α_{2u}-globulin accumulation were done (Table 4-8). Although no significant increase in labeling index in the renal cortex was determined by standard assessment methods, focal areas of increased BrdU labeling were noted. Quantitation of these areas revealed increased cell proliferation in subcapsular proximal tubules (cortex 1) in animals exposed to THF at the mid and high concentration for 20 days and at the high concentration for 5 consecutive days. No increase in labeling was observed in the groups given a 21-day recovery period. An increase in cell proliferation was also noted in the proximal tubules between the outer stripe of the outer medulla and the subcapsular layer (cortex 2) at the highest concentration following 20 exposures.

Table 4-8. Mode-of-action studies in male rat kidneys

Exposure protocol	Control		600 mg/m^3		1800 mg/m^3		5400 mg/m^3	
	%	LC (M)[a]	%	LC (M)	%	LC (M)	%	LC (M)
5 Exposures								
BrdU labeling: cortex 1	100	112	95	107	109	122	153[b]	171
BrdU labeling: cortex 2	100	132	102	134	99	131	125	165
TUNEL: whole cortex	100	13[c]	115	15	107	14	92	12
5 Exposures + 3 weeks recovery								
BrdU labeling: cortex 1	100	138	78[d]	107	88	121	110	152
BrdU labeling: cortex 2	100	140	86	121	86	120	105	147
TUNEL: whole cortex	100	9	45	4	145	13	478[b]	43
20 Exposures								
BrdU labeling: cortex 1	100	118	119	140	159[b]	188	298[b]	352
BrdU labeling: cortex 2	100	156	101	158	113	176	186[b]	290
TUNEL: whole cortex	100	35	74	26	157	55	234[b]	82
Exposure protocol	**Control**		**600 mg/m^3**		**1800 mg/m^3**		**5400 mg/m^3**	
	%	**LA (%)[e]**	**%**	**LA (%)**	**%**	**LA (%)**	**%**	**LA (%)**
5 Exposures								
α_{2u}-globulin: whole cortex	100	6.16	136[d]	8.37	171[b]	10.53	178[b]	10.95
α_{2u}-globulin: cortex 1	100	7.30	125	9.14	167[b]	12.18	175[d]	12.75
α_{2u}-globulin: cortex 2	100	5.01	131	6.57	176[b]	8.82	188[b]	9.42
5 Exposures + 3 weeks recovery								
α_{2u}-globulin: whole cortex	100	5.57	150	8.35	212[b]	11.80	299[b]	16.66
α_{2u}-globulin: cortex 1	100	6.68	154	10.32	213[b]	14.22	280[b]	18.70
α_{2u}-globulin: cortex 2	100	4.47	141	6.30	205[d]	9.18	324[b]	14.49
20 Exposures								
α_{2u}-globulin: whole cortex	100	5.34	149[d]	7.97	221[b]	11.79	259[b]	13.84
α_{2u}-globulin: cortex 1	100	6.20	149[b]	9.21	212[b]	13.15	253[b]	15.70
α_{2u}-globulin: cortex 2	100	4.47	149[d]	6.66	236[b]	10.53	265[b]	11.86

[a]LC (M) = positively labeled cells (LCs) mean value.
[b]$p \leq 0.01$.
[c]Number of apoptotic cells.
[d]$p \leq 0.05$.
[e]LA (%) = percent of labeled area (LA).

Source: Adapted from BASF (2001a).

To determine whether changes in cell growth might reflect altered apoptosis rates, apoptotic cells were also quantitated (Table 4-8). The number of cells undergoing apoptosis was significantly increased in the high-concentration groups exposed for 5 days and observed for 21 days or after 20 exposure days. Marginal increases were observed in the mid-concentration groups for these two exposure regimens, but the results were not statistically significant. The authors suggested the increase in apoptosis observed in the group with a recovery period might

be greater than in the 20-day exposure group, because in the latter group competing cell proliferation and apoptosis events might have reduced the degree of apoptosis.

THF exposure also induced α_{2u}-globulin accumulation in male rats treated under all three of the separate exposure regimens (Table 4-8). Increases were generally concentration related, with increases at the high concentration ranging from 175% to 280% of control levels for cortex 1 and from 188% to 324% of control levels for cortex 2, among the three exposure regimens. When the whole cortex was used as the labeled area for the analysis, accumulation was significantly elevated beginning at the low concentration following 5 consecutive days or 20 days of exposure. Maximum effects observed at the high concentration ranged from 178% to 299% of controls among the three exposure regimens. The accumulation of α_{2u}-globulin as measured by the immunohistochemical staining technique was supported by histopathological evaluation of control and high-concentration animals exposed to THF for 20 days. The incidences of proximal tubule cells with grade 2 (slightly increased) staining for hyaline droplets were 1/6 and 5/6 for controls and high-concentration animals, respectively. THF exposure had no effect on CYP450 content or CYP450 enzyme activities in the male rat kidneys.

No clinical effects or gross pathology changes related to the THF exposures were reported for female mice. In mice exposed for 5 days, absolute and relative uterus weights were decreased in the high-dose group. In mice exposed for 5 days and followed for a 21-day recovery period, relative uterus weights were decreased (up to 21%) and appeared to decrease in a concentration-dependent manner, although this decrease was not statistically significant. In mice exposed 20 days, statistically significant increases in absolute body weight (5%), absolute liver weight (11%), and relative liver weight (6%) were reported. The absolute and relative uterus weights were decreased by 11% and 15%, respectively. None of the uterus weight changes for any of the groups were statistically significant. No treatment-related histopathological effects were observed in the uterus at any concentration. No clearly adverse histopathological effects in the liver were reported by the study authors. However, some treatment-related effects were noted. One effect that appeared to have some relationship with THF exposure was fatty phanerosis (unmasking of previously invisible fat in the cytoplasm). In animals exposed for 5 consecutive days at the mid concentration, fatty phanerosis was observed in 5 of 10 animals. Effects were more pronounced in the high-concentration group, where fatty phanerosis in zones 3 and 2 (midzonal) was observed in all the exposed mice. In the group exposed for 5 days, further histological analysis of two randomly selected animals identified minimal fatty infiltration in livers from mice exposed to the mid and high concentrations, although signs of degeneration were not observed. Other histopathological changes in the high-concentration group included a change in the appearance of the hepatocyte cytoplasm to a more homogeneously eosinophilic appearance as compared with hepatocytes in control livers. Histological changes in mice exposed for 5 days then allowed a 21-day recovery period were less notable, suggesting reversibility of the effects. Under this treatment regimen, fatty phanerosis

was reported in one high-concentration animal. Fatty phanerosis was detected in all animals in the high-concentration group exposed for 20 days; however, this effect did not appear to be concentration related. No degenerative changes were noted.

THF exposure increased cell proliferation (Table 4-9) in the female mouse liver. Since chemical exposures can have varying effects in different regions of the liver lobule, cell proliferation was evaluated separately for zone 1 (the region adjacent to the portal triad), zone 3 (the region adjacent to the central vein), and zone 2 (the area of the lobule intermediate between zones 1 and 3). Increased cell proliferation was observed in zones 2 and 3 of the liver following THF exposure for 5 days and in zone 3 following 20 exposures. No concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period, suggesting that the increases in cell proliferation may be an adaptive effect. Coincident with the increase in BrdU labeling, the mitotic index was increased in zone 3 after 5 or 20 exposures in the high-concentration groups. No treatment-related change in the number of liver cells undergoing apoptosis was observed. The number of stained cells was small, suggesting that THF did not induce an apoptotic response under the exposure conditions. Five consecutive days of exposure to THF at the high concentration generated a statistically significant increase in CYP450 content in the liver (125% of controls; $p \leq 0.05$), EROD activity (192% of controls; $p \leq 0.01$), and PROD activity (321% of controls; $p \leq 0.05$).

Table 4-9. Mode-of-action studies in female mouse livers

Exposure protocol		Control		600 mg/m^3		1800 mg/m^3		5400 mg/m^3	
5 Exposures									
BrdU labeling (% of control)		**%**	**LI[a] (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	1.01	110	1.11	122	1.23	143	1.44
	Zone 2	100	2.54	98	2.48	117	2.96	183[d]	4.66
	Zone 3	100	0.85	147	1.25	188	1.60	401[d]	3.41
	Zone 1,2,3	100	1.46	110	1.61	132	1.93	217[d]	3.17
Hematoxylin and eosin: mitotic index (MI)		**MI[b] (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.01		0.01		0.03		0.04	
	Zone 2	0.14		0.14		0.17		0.48[d]	
	Zone 3	0.00		0.01		0.00		0.19[c]	
	Zone 1,2,3	0.05		0.05		0.07		0.23[d]	
5 Exposures + 3 week recovery									
BrdU labeling (% of control)		**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	0.88	120	1.06	100	0.88	109	0.96
	Zone 2	100	2.75	107	2.95	85	2.35	76	2.08
	Zone 3	100	1.09	170[d]	1.85	148	1.61	137	1.49
	Zone 1,2,3	100	1.57	124	1.95	103	1.61	96	1.51
Hematoxylin and eosin: mitotic index (MI)		**MI (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.00		0.00		0.01		0.00	
	Zone 2	0.02		0.01		0.04		0.08	
	Zone 3	0.00		0.00		0.04		0.03	
	Zone 1,2,3	0.01		0.00		0.03		0.04	
20 Exposures									
BrdU labeling (% of control)		**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**	**%**	**LI (%)**
	Zone 1	100	1.39	106	1.48	91	1.27	104	1.45
	Zone 2	100	3.53	86	3.02	95	3.35	118	4.16
	Zone 3	100	1.52	133	2.02	134	2.04	230[d]	3.49
	Zone 1,2,3	100	2.51	101	2.17	103	2.22	141	3.03
Hematoxylin and eosin: mitotic index (MI)		**MI (%)**		**MI (%)**		**MI (%)**		**MI (%)**	
	Zone 1	0.05		0.05		0.01		0.05	
	Zone 2	0.04		0.16		0.32[c]		0.24[d]	
	Zone 3	0.01		0.01		0.07		0.20[d]	
	Zone 1,2,3	0.03		0.07		0.13[c]		0.16[d]	

[a]LI = labeling index.
[b]MI = mitotic index.
[c]$p \leq 0.05$.
[d]$p \leq 0.01$.

Source: Adapted from BASF (2001a).

In mice, BrdU labeling in the uterine epithelium of the controls was high; however, no statistically significant changes in this measure were detected for any of the treatment groups.

Also, the mitotic index was not significantly affected by THF exposure, while the percent increase in mitotic index was large for mice exposed to the highest concentration for 5 days followed by a 21-day recovery. The authors (BASF, 2001a) suggested that an unusually low number of mitotic cells identified in the control animals contributed to the apparent increase in mitosis. The number of apoptotic cells was increased (168% of controls) in the high-concentration group given a 21-day recovery period. However, the overall data do not suggest that apoptosis plays a major role in cell regulation by THF, since the corresponding concentration in groups exposed 5 or 20 days had no increase in apoptosis (TUNEL staining). In addition, the total number of stained cells was small, suggesting that THF does not induce a robust apoptotic response in the uterus.

The study authors (Gamer et al., 2002; BASF, 2001a) discussed the implications of their mode-of-action studies for use in identifying the tumor mode of action for male rat kidney tumors and liver tumors in female mice. For male rats, the concordance between α_{2u}-globulin accumulation, cell proliferation, and induction of apoptosis in the renal cortex, with doses that induce kidney tumors in cancer bioassays, appears to support the involvement of these mechanisms in THF-induced rat kidney tumors. The detection of α_{2u}-globulin accumulation only when sensitive detection methods were used (i.e., immunohistochemical staining as opposed to standard staining for histopathological examination) suggests that the responses are weak. Furthermore, the observed cell proliferation response, which was increased to a maximum of 298% of controls when selected for focal areas of proliferation, was minimal as compared with cell proliferation responses induced by other well-characterized inducers of α_{2u}-globulin accumulation (U.S. EPA, 1991b). For the female mouse livers, the authors conclude that the THF may act by inducing cell proliferation, based on the observed increase in liver weight, BrdU labeling, and mitotic index. Although some histological changes were noted, including fatty phanerosis and an altered texture of the cytoplasm in zones 3 and 2 (more homogeneous and eosinophilic), no gross degenerative histopathology such as necrosis was found.

CYP450 activity was also evaluated as part of a mode-of-action study to identify the tumorigenic mode of action for THF (Gamer et al., 2002; BASF, 2001a). Female B6C3F1 mice were exposed nose only to average THF concentrations of 0, 598, 1811, or 5382 mg/m^3 (0, 199, 604, or 1794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Five consecutive days of exposure to THF at the high concentration generated a statistically significant increase in CYP450 content in the liver (125% of controls; $p \leq 0.05$), EROD activity (192% of controls; $p \leq 0.01$), and PROD activity (321% of controls; $p \leq 0.05$). EROD activity is often used as a measure of CYP1A family activity, while PROD is often used as a measure of CYP2B family activity, although there is some overlap in the specificity of these assays for various CYP450 isoforms among species (Weaver et al., 1994). This result would suggest that THF might be metabolized by CYP1A/2B isoforms, although these data do not provide direct evidence of their involvement.

Female B6C3F1 mice were exposed to THF concentrations of 0, 5512, or 14,739 mg/m^3 6 hours/day for five consecutive days (BASF, 2001b). The target concentrations of 5400 and 15,000 mg/m^3 were chosen to match the high-concentration groups in the subchronic NTP (1998) study. Two groups of mice were used for each THF concentration. One group of mice was pretreated with an i.p. dose of 100 mg/kg 1-aminobenzotriazole (ABT), a potent inhibitor of CYP450 enzyme activity that has broad activity for many CYP450 isoforms. The parallel exposure group did not receive this pretreatment with ABT and was used to test the effects of THF without CYP450 inhibition. The livers of the mice were evaluated for the activity of CYP450s (as measured by total CYP450 content, EROD activity, PROD activity, and nitrophenol hydroxylase activity), cell proliferation (as measured by PCNA staining), and examination by electron microscopy.

Exposure of animals at the high concentration induced a narcotic effect. Three of 18 mice died in the high-concentration group without CYP450 inhibition, and 1 of 18 mice died in the high-concentration group pretreated with ABT. The high-concentration group mice also had reduced body weights compared with controls. No clinical effects of THF were observed at the low concentration. No THF-related histopathology changes were observed in any of the treatment groups, although, in the livers of ABT-pretreated mice, centrilobular fatty changes were noted. Measurements of CYP450 content and activity revealed that CYP450s were induced in the high-concentration mice. Liver CYP450 content was increased by 98% in the high-concentration group, and this increase was blocked by ABT pretreatment. THF treatment induced PROD activity by about sixfold in the high-concentration group. In the mice pretreated with ABT, PROD activity was induced by approximately twofold by THF. EROD activity was increased by 160% in the high-concentration group mice as compared to controls in the absence of ABT, and no induction of EROD activity was observed in the mice pretreated with ABT. These results show that THF induces both EROD and PROD activity and that the ABT pretreatment was an effective inhibitor of CYP450 isoform activity. In contrast to the results for PROD and EROD, nitrophenol hydroxylase activity was decreased in a concentration-dependent manner by THF and was not affected by ABT pretreatment. CYP450 content or associated enzyme activities were not induced above basal levels in the low-concentration group.

THF exposure induced cell proliferation at the high concentration, regardless of pretreatment with ABT. In mice exposed to 14,739 mg/m^3 THF without ABT pretreatment, PCNA staining was increased 814% relative to controls in zone 3, although a decrease to 59% of control levels that was not statistically significant was observed in zone 2, and no difference was observed for zone 1. The overall increase in PCNA staining for the three zones (pooled data) was 133% of controls (not statistically significant). In the high-concentration group pretreated with ABT, cell proliferation was even greater than the parallel THF group without pretreatment. PCNA staining was 150%, 280%, and 1050% of control levels in liver zones 1, 2, and 3, respectively. In ABT-pretreated mice, the overall PCNA labeling for the three zones (data

pooled) was 329% of controls. No change in PCNA staining was observed in the low-concentration groups regardless of pretreatment with inhibitor.

The data indicated that THF is an inducer of CYP450s and that THF induces cell proliferation in the livers of female mice, particularly in zone 3 hepatocytes. Pretreatment with the CYP450 inhibitor ABT enhanced the degree of PCNA staining, suggesting that THF itself, rather than a downstream oxidative metabolite, is responsible for the cell proliferative response. In mice with enzyme inhibition the cell proliferation response was enhanced only moderately. It is possible that this effect would have been even more dramatic if the basal as well as inducible CYP450 activity had been blocked by the ABT pretreatments. ABT did not provide a complete inhibition of response, producing some uncertainty about the role that CYP450s play in THF-induced cell proliferation. A second area of uncertainty is that there were qualitative differences in the histopathology in the ABT-pretreated mice (i.e., centrilobular fatty changes) compared to mice without ABT pretreatment. It is not clear whether these histopathological changes that were unique to ABT-pretreated mice could have caused hepatocytes to be more susceptible to THF-induced liver toxicity. Even though these areas of uncertainty remain, the most plausible interpretation of the data is that the cell proliferative response of the liver in female mice is not dependent on CYP450 activity, since treatment with the CYP450 inhibitor did not decrease the proliferative response. This interpretation suggests that THF itself, not a metabolite, is the active moiety in inducing cell proliferation.

Other than the NTP (1998) study, no direct animal cancer bioassays have been conducted. The use of THF as a solvent control in cancer studies for other compounds provides some limited data on the potential cancer mode of action for THF. Sawyer et al. (1988) evaluated the tumor-initiating properties of dibenz[a,j]anthracene, cholanthrene, and their diol and epoxide metabolites on the skin of SENCAR mice. The test compounds were dissolved in either acetone (30 mice/group) or THF (24 mice/group). The number of papillomas per mouse and percent of mice with papillomas was lower for THF-treated controls (5%) than for acetone-treated controls (16%) and was much lower than for the animals treated with the test compounds (39–97% for various treatment groups), suggesting that THF is not a potent tumor initiator. However, interpretation of this study is limited for a number of reasons. The study authors did not provide data on the historical incidence of papillomas. A tumor-screening protocol was used, which did not include a control group, an adequate number of dose levels, or adequate numbers of animals per dose group. Another complication in evaluating this study is that the tumor incidences for the test compounds dissolved in acetone or THF could reflect cocarcinogenic interactions.

Chen et al. (1984) investigated the ability of organic solvents to inhibit gap junctional intercellular communication (GJIC). Cocultures of 6-thioguanine-sensitive and resistant Chinese hamster V79 fibroblast cells were treated with the test compound and the degree of metabolic cooperation was determined by the survival of the resistant cells. The killing of resistant cells

serves as an indicator of metabolic cooperation, because the toxic 6-thioguanine metabolite that is formed only in the sensitive cells can be passed on to normally resistant cells when gap junctions are intact. Therefore, robust growth of the resistant cells in this assay system would suggest that GJIC is inhibited. THF was judged to be positive (as defined by a doubling in recovery of resistant colonies) in the metabolic cooperation assays, suggesting that THF can inhibit GJIC. The recovery rate of resistant cells increased with increasing concentration (up to 100 μL of THF/5 mL of medium). Although the mechanism is unclear, there appears to be a correlation between inhibition of GJIC and mouse liver carcinogenesis by some nongenotoxic carcinogens (Klaunig et al., 1998).

4.4.4. Genotoxicity Studies

Only one study that evaluated genotoxicity endpoints in humans was identified. Funes-Cravioto et al. (1977) reported increased chromosome breaks in peripheral lymphocytes from solvent-exposed versus nonexposed adults. However, of the seven occupational groups that were pooled for the statistical analysis, only one was identified as having used THF in the workplace (no exposure information was provided by the study authors), thus suggesting that agents other than THF likely played a greater role in the observed genotoxicity.

NTP (1998) presented the results of a battery of genotoxicity tests of THF. The in vitro tests included the *Salmonella typhimurium* bacterial mutagenicity assay (with and without S9 microsomal activation), induction of sister chromatid exchange and chromosomal aberrations in the Chinese hamster ovary cell system, and in vivo in mouse bone marrow cells. Micronuclei frequency in peripheral blood erythrocytes following 14-day inhalation exposure of mice to THF was also evaluated. NTP (1998) concluded that there was little evidence of mutagenic activity, with most data determined to be conclusively negative. The genotoxicity data are summarized in Table 4-10 and discussed in more detail below.

Table 4-10. Summary of genotoxicity studies

Endpoint	Assay system	Results (wo/w activation)	Comments	Reference
In vitro studies				
Gene mutation—bacteria	*S. typhimurium* TA1535, TA1537, TA98, TA100	–/–	Used preincubation modification of the standard assay (NTP [1998] study).	Mortelmans et al. (1986)
	S. typhimurium G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98, *Escherichia coli* WP2, WP2 *uvrA*$^-$	–/–	Gradient technique was used in which the mutagenic concentration range was identified as the lowest and highest concentration at which distinct colonies were observed. Results presented in a summary table without data.	McMahon et al. (1979)
	S. typhimurium TA1535, TA1537, TA98, TA100	–/–	Screening only using a spot test was done in strains TA1535, TA1537, TA98. Results presented in a summary table without data.	Florin et al. (1980)
	S. typhimurium TA98	nt[a]/–	Results presented in summary text without data.	Arimoto et al. (1982)
Clastogenicity	Micronuclei, Syrian hamster embryo cells	nt/–	None.	Gibson et al. (1997)
Chromosome aberration	Chinese hamster ovary cells	–/±	Slight increase with S9 not considered positive by study authors. NTP study (1998).	Galloway et al. (1987)
DNA damage	Sister chromatid exchange, Chinese hamster ovary cells	–/–	NTP study (1998).	Galloway et al. (1987)
Cell transformation	BALB/c-3T3 cells	–/nt	Limited activity was noted in one of two trials in the data tables, but not in the text of the study.	Matthews et al. (1993)
	Syrian hamster embryo cells	–/nt	No cytotoxicity was observed at the highest test concentration.	Kerckaert et al. (1996)
	NIH/3T3 cells	–/nt	THF used as control. Cells treated in vitro were injected in mice to assess tumorigenicity.	Collins et al. (1982)
In vivo studies				
Gene mutation	Drosophila sex-linked recessive lethal	–	NTP (1998) study.	Valencia et al. (1985)
Clastogenicity	Mouse erythrocyte micronucleus	±	Positive response only in mid-concentration males (NTP [1998] study).	NTP (1998)
Chromosome aberration	Mouse bone marrow	–	NTP (1998) study.	NTP (1998)
DNA damage	Mouse bone marrow, sister chromatid exchange	–	NTP (1998) study.	NTP (1998)
	Mouse hepatocyte unscheduled DNA synthesis	–	NTP (1998) study.	Mirsalis et al. (1983)

[a]nt = not tested.

Mortelmans et al. (1986) reported that THF did not induce reverse mutations with or without metabolic activation in four tester strains of the *S. typhimurium* test system. THF was also negative (with or without activation) when tested in a battery of eight strains of *S. typhimurium* and two *Escherichia coli* strains by using a modification of the standard assay (McMahon et al., 1979) or in four *S. typhimurium* strains (Florin et al., 1980). Several studies used or specifically examined the effects of THF as a soluble solvent in the *S. typhimurium* mutagenicity assays and generally support the conclusions of the above-mentioned more definitive studies. Hageman et al. (1988), in a study of the mutagenicity of frying oils, reported that THF solvent controls were nonmutagenic (with or without activation) in tester strains TA97, TA100, and TA104 relative to mutagen-containing oil samples. Maron et al. (1981) screened a series of solvents for compatibility with the *S. typhimurium* test system and reported that, while high-dose THF was toxic to the four tester strains used, it did not affect the mutagenicity of benzo(a)pyrene at lower levels (50 μL/plate) in strain TA100 in the plate incorporation protocol. THF was judged to be an unsatisfactory solvent for the preincubation assay due to higher cytotoxicity observed in this protocol modification. Finally, THF was reported to enhance the mutagenicity of tryptophan pyrolysate mutagens in *S. typhimurium* preincubation assay when used as a solvent (Arimoto et al., 1982). No potential mode of action for this effect was given, but the authors reported (no quantitative data provided) that the solvent was not itself mutagenic in tester strain TA98 with activation. The studies by Hageman et al. (1988), Maron et al. (1981), and Arimoto et al. (1982) are of limited value for assessing the mutagenic potential of THF because THF served as the control solvent in these studies and it is not clear if the results for THF were compared to untreated samples.

THF was also negative in a variety of in vitro assays evaluating chromosome and DNA damage up to cytotoxic concentrations. Gibson et al. (1997) reported that THF did not increase micronuclei formation when assayed in Syrian hamster embryo cells at concentrations that significantly reduced cell number. Galloway et al. (1987) reported some increase in total chromosome aberrations in the presence of S9 activation in Chinese hamster ovary cells. A majority of the aberrations were classified as simple, including breaks and terminal deletions. The study authors suggested that these increases were insufficient to be scored as a positive result. As part of this same study, Galloway et al. (1987) reported that THF did not induce sister chromatid exchanges in this cell system at cytotoxic doses.

THF was judged to be inactive when tested in the standard BALB/c-3T3 mouse cell transformation assay (Matthews et al., 1993). A Syrian hamster embryo cell assay was also negative for cell transformation when THF was tested at concentrations up to 5 mg/mL (Kerckaert et al., 1996). Collins et al. (1982) evaluated the in vivo tumorigenicity of NIH/3T3 cells transformed in vitro by benzo[a]pyrene-trans-7,8-dihydrodiol-9,10-epoxide (BPE) dissolved in THF. The ability of BPE-treated cells to induce tumors in normal mice (strain not specified) and AT×FL mice having a compromised immune response (thymectomized, lethally

irradiated, and restored with syngeneic liver cells) was greater than the tumorigenicity of cells treated with THF only. Cells from 46/57 BPE-treated plates were tumorigenic in vivo, whereas cells from only 2/20 of the THF-treated plates were tumorigenic when injected in mice. The background tumor rate for untreated mice was not reported, but the low incidence of tumors induced by THF-treated cells as compared with positive controls suggested that THF did not significantly increase the rate of cell transformation.

THF has also generated negative findings in in vivo genotoxicity assays. THF did not induce sex-linked recessive lethal mutation in *Drosophila melanogaster* in a screening test for 48 chemicals for NTP (Valencia et al. 1985). NTP (1998) evaluated the formation of micronuclei in peripheral blood erythrocytes in male and female mice at the end of their 13-week inhalation study. There was only a statistically significant increased incidence of micronucleated normochromatic erythrocytes at the mid concentration in males. The effect was not concentration dependent, and no corresponding increase was seen for females. The results were considered by NTP to be equivocal. In a bioassay for chromosomal aberrations, male B6C3F1 mice received THF by i.p. injection at doses of up to 2000 mg/kg. No significant increase in the number of aberrations per cell or percent of bone marrow cells with aberrations was observed (NTP, 1998).

In vivo assays for DNA damage have also been conducted for THF. Male B6C3F1 mice received THF doses of up to 2000 mg/kg by i.p. injection. Bone marrow cells were harvested after 23 or 42 hours of exposure. In the 23-hour treatment protocol, a significant increase in the mean number of sister chromatid exchanges per cell was reported for the high-dose animals. However, this effect was observed in only one of the two replicate trials. No increase in sister chromatid exchanges was reported for the animals exposed for 42 hours. NTP (1998) characterized these results as negative. In another assay for DNA damage, Mirsalis et al. (1983 [published abstract]) reported that in vivo treatment of male rats with THF did not induce unscheduled DNA synthesis in hepatocytes.

In summary, the genotoxic potential of THF has been evaluated in a variety of in vitro and in vivo assays. Nearly all the results are conclusively negative, with equivocal findings reported in only a small number of assays that have been conducted. Taken together, these data support the conclusion that THF is not genotoxic.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

The oral database for THF is very limited. A summary of the effects observed in the subchronic oral studies is presented in Table 4-11. No human studies of THF in oral exposure are available. In rats treated with a total of six gavage doses of THF in distilled water, increased mortality was observed at doses greater than 2000 mg/kg (Stasenkova and Kochetkova, 1963). Toxicity observed in this study included CNS toxicity (immobility, drowsiness, reduced response

to external stimuli) and necrosis, edema, and hemorrhage of stomach, brain, liver, heart, spleen, and kidneys. In a 4-week study of THF in drinking water administered to rats (Komsta et al., 1988), doses as high as 96 mg/kg-day had no effect on mortality nor produced clinical signs of CNS toxicity in rats. Histopathologic lesions in liver (increased cytoplasmic homogeneity and anisokaryosis) and kidney (tubular cytoplasmic inclusions) were observed in the high-dose group males and females. In a one-generation reproductive toxicity study (BASF, 1994) of THF administered to rats in drinking water, symptoms of general toxicity, including decreased food (males) and water consumption (males and females) and increased kidney weight (males and females), were observed in parental generation rats administered 8000 ppm THF (795 mg/kg-day for males and 890 mg/kg-day for females). At this concentration, male and female pups had significantly decreased body weights compared with controls. A follow-up two-generation reproductive toxicity study (BASF, 1996) of THF administered to rats in drinking water demonstrated similar results as the one-generation study in the parental generation rats, including decreased food consumption (F0 females, F0/F1 males), decreased water consumption (F0/F1 both sexes), decreased body weight (F0/F1 both sexes), and increased kidney weight (F0 both sexes) at 9000 ppm THF (714 mg/kg-day for F0 males, 788 mg/kg-day for F1 males, 835 mg/kg-day for F0 females, 898 mg/kg-day for F1 females). At these same concentrations, the F1 and F2 pups had significantly reduced body weights compared with controls, and the F2 pups also demonstrated delayed opening of eyes and ear canal compared with controls (see Table 4-7). Limited histopathology examination on parental rats included liver, kidney, reproductive organs, and digestive tract organs and demonstrated no observed effects on these organs. In both studies, no effects were observed on any other reproductive parameters measured.

Table 4-11. Summary of oral toxicity studies of THF

Study	Species, number, sex	Route, duration, doses	Observed effects	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Comments
Komsta et al. (1988)	Sprague-Dawley rat 10/sex/group	Drinking water, 4 weeks 0, 0.1, 0.8, 10, 96 mg/kg-day	Histopathological, lesions in liver (males and females) and kidney (females)	Not identified	96	Histopathology only done on high-dose group, no statistics—high dose may not be significant, short duration
BASF (1996)	Wistar rat 25/sex/dose	Drinking water, two-generation reproductive 0, 1000, 3000, 9000 ppm	General toxicity in F0 and F1 adults, decreased body weight in F1/F2 pups, delayed eye opening in male F2 pups	See Table 4-7[b]	See Table 4-7[b]	Each generation treated 70 days prior to mating through cohabitation, gestation, lactation; essentially a subchronic study
BASF (1994)	Wistar rat 10/sex/dose	Drinking water, one-generation reproductive 0, 4000, 8000, 12,000 ppm	Increased kidney weight (F0 males—high dose, F0 females—mid dose) Decreased pup body weight (mid dose)	503 546	890 960	

[a]The best value to use for estimating the corresponding doses (mg/kg-day) differs for each generation based on substance intake values over the relevant period of exposure. For parental effects, average substance intakes over the entire study period are appropriate for use in assigning effect levels. For developmental effects, the time-weighted average substance intake during the gestation and lactation periods of the parent females was used to estimate the effective dose. Substance intake estimates are shown in Table 4-4.
[b]Substance intake estimates corresponding to NOAEL and LOAEL estimates were calculated for a variety of effects and are presented in Table 4-7.

4.5.2. Inhalation

A summary of the effects observed in the subchronic and chronic studies is presented in Table 4-12. Although no epidemiological studies of THF have been conducted, several case studies in humans illustrate the potential for health effects following inhalation exposure in an occupational setting. In almost all of the cases, workers were exposed to THF through activities where THF was present as a component of solvents or adhesives. In general, workers were exposed for a period of a few weeks to a few months before symptoms were reported. Target organs in humans appear to be the CNS, respiratory tract, liver, and kidney. Symptoms of CNS toxicity included headache, dizziness, fatigue, loss of the sense of smell (Garnier et al., 1989; Emmett, 1976; Horiuchi et al., 1967), and convulsions following enfluran anesthesia in a worker exposed to THF in the weeks prior to surgery (Juntunen et al., 1984). Symptoms of respiratory tract irritation included cough, chest pain, rhinorrhea, and dyspnea (Garnier et al., 1989; Emmett, 1976). In three cases, liver enzymes (ALT, AST, and GGT) were elevated above normal values (Garnier et al., 1989; Edling, 1982; Horiuchi et al., 1967), and in one case a liver biopsy revealed fatty changes following THF exposure (Edling, 1982). In one study, hematological changes and decreased white blood cell counts were reported in THF-exposed workers (Horiuchi et al., 1967). In one case study, autoimmune glomerulonephritis was observed in a man who worked with THF in adhesives for 9 years (Albrecht et al., 1987). Thus, the human case studies suggest that CNS toxicity, respiratory tract irritation, and liver and kidney toxicity are the potential health effects following inhalation exposure to THF. An uncertainty associated with all of the reported human case studies is the fact that workers were exposed to other solvents and chemicals in addition to THF, so it is not possible to conclusively attribute the observed effects to THF exposure. In addition, in most cases quantitative estimates of exposure were not provided. Based on these two considerations, the human data are insufficient to serve as the basis for derivation of an RfC.

Table 4-12. Summary of subchronic and chronic inhalation studies of THF						
Study	**Species, sex, number, concentration (mg/m^3)**	**Duration**	**Observed effects**	**NOAEL/ LOAEL[a] (mg/m^3)**	**NOAEL/ $LOAEL_{HEC}$ (mg/m^3)**	**Comments**
Subchronic Studies						
BASF (1938)	Dog, sex and strain not specified (four/group)	590 mg/m^3: 6 hours/day, 5 days/week, 9 weeks then 1080 mg/m^3: 6 hours/day, 5 days/week, 3 weeks	Decreased blood pressure	NA[b]/590	NA/105	No microscopic pathology noted in heart, lungs, spleen, pancreas, or kidneys
Horiguchi et al. (1984)	Sprague-Dawley rat, male (11–12/group) 0, 295, 590, 2950, 14,750	4 hours/day, 5 days/week, 12 weeks	Body and organ weight changes, altered serum chemistry	2950/14,750	351/1726	
Kawata and Ito (1984)	Wistar rat, male (25/group) 0, 8850	1 hour/day, 5 days/week, 12 weeks	Decreased body weight, papillary hyperplasia in lung and bronchial epithelium, protein casts/hyaline in kidney	NA/8850	NA/ HECTB = 400 HECPU = 490 HECER = 263	No information given on incidence of histopathologic lesions or statistical significance
DuPont Haskell Laboratory (1996b)	Crl:CD BR (12–18/sex/group) 0, 1475, 4425, 8850	6 hours/day, 5 days/week, 13–14 weeks	CNS clinical signs	1475/4425	263/790	This was a subchronic neurotoxicity study. No other neurotoxic effects were observed (i.e., FOB, motor activity, or neuropathology)
NTP (1998)	F344 rat (10/sex/group) 0, 195, 590, 1770, 5310, 14,750	6 hours/day, 5 days/week, 90 days	CNS clinical signs, organ weight changes, hematological effects	5310/14,750	948/634	
	B6C3F1 mouse (10/sex/group) 0, 195, 590, 1770, 5310, 14,750	6 hours/day, 5 days/week, 90 days	CNS clinical signs, increased liver weight	1770/5310	316/948	Decreased thymus weight at lower concentrations and histopathology of the liver, uterus, adrenal gland only at the high concentration
Chronic Studies						
Stasenkova and Kochetkova (1963)	Rat, male, strain not specified (20/group) 1000–2000	4 hours/day, 7 days/week, 6 months	Decreased blood pressure, increased leukocyte count, hypertrophy of muscle fibers in bronchi walls and spleen	NA/NA	NA/NA	Air concentration reported as a range; study judged as not suitable for dose-response assessment

Table 4-12. Summary of subchronic and chronic inhalation studies of THF

Study	Species, sex, number, concentration (mg/m^3)	Duration	Observed effects	NOAEL/ LOAEL[a] (mg/m^3)	NOAEL/ $LOAEL_{HEC}$ (mg/m^3)	Comments
NTP (1998)	F344 rat (50/sex/group) 0, 590, 1770, 5310	6 hours/day, 5 days/week, 2 years	No noncancer effects observed	5310/NA	948/NA	
	B6C3F1 mouse (50/sex/group) 0, 590, 1770, 5310	6 hours/day, 5 days/week, 2 years	CNS clinical signs (males)	1770/5310	316/948	Decreased survival, urogenital tract inflammation and histopathology lesions in bone marrow, lymph nodes, spleen, thymus attributed to infection secondary to observed narcosis
Reproductive and Developmental Toxicity Studies						
Mast et al. (1992)	CD-1 mice, female (40/group) 0, 1770, 5310, 14,750	6 hours/day, 7 days/week, gestation days 6–17	Decreased dam body weight and gravid uterine weight, decreased fetal survival	1770/5310	433/1328	
	Sprague-Dawley rat, female (40/group) 0, 1770, 5310, 14,750	6 hours/day, 7 days/week, gestation days 6–19	Decreased dam body weight, decreased fetal body weight	5310/14,750	1328/3688	
DuPont Haskell Laboratory (1980)	Crl:CD BR rat, female (29/group) 0, 590, 1475, 2950, 7375, 14,750	6 hours/day, 7 days/week, gestation days 6–15	Dams: CNS clinical signs Fetal: decreased fetal weight, skeletal alterations	Dams: 1475/2950 Fetal: 7375/14,750	369/738	

[a]NOAEL/LOAEL from the study concentrations.
[b]NA indicates that the NOAEL or LOAEL was not assigned.

Several acute inhalation studies in animals suggest that the primary effects observed following single exposures to THF, ranging from 30 minutes to several hours, are CNS toxicity and respiratory tract irritation. Symptoms of CNS toxicity, including sedation, coma, altered respiration, and decreased response to external stimuli, were observed in dogs (Stoughton and Robbins, 1936), mice (Stasenkova and Kochetkova, 1963; Stoughton and Robbins, 1936), and rats (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979; Stasenkova and Kochetkova, 1963). Clinical signs of respiratory tract irritation, observed only in studies in rats, included scratching, head shaking, face washing, tearing, salivation, and bleeding from the nose (Horiguchi et al., 1984; DuPont Haskell Laboratory, 1979). In addition, several other acute studies observed structural or functional changes in respiratory tissue (suggesting respiratory tract irritation), including congested mottled lungs in rats (Henderson and Smith, 1936), edema and hemorrhage in lungs and bronchi of rats (Stasenkova and Kochetkova, 1963), and decreased ciliary beat frequency and vacuolation/degeneration of both nasal mucosa (Ohashi et al., 1983) and tracheal mucosa (Ikeoka et al., 1988) in rabbits, and nasal and tracheal histopathology changes in rats (Horiguchi et al., 1984). Two studies report histopathological lesions in other organs such as liver (Henderson and Smith, 1936), kidney, brain, and spleen (Stasenkova and Kochetkova, 1963). However, Hofmann and Oettel (1954) specifically examined the liver and kidney and found no effects.

As with acute exposure, longer-term inhalation exposure to THF also appears to result in symptoms of CNS toxicity and respiratory tract irritation. However, these symptoms appear to moderate as exposure duration increases, suggesting either that the animals develop a tolerance to these effects or that THF induces enzymes that increase the metabolism of THF to a less toxic compound. In two subchronic studies, authors specifically note that symptoms of CNS toxicity (NTP, 1998; Horiguchi et al., 1984) and/or local irritation (Horiguchi et al., 1984) appeared to moderate with continued exposures. In addition, Stasenkova and Kochetkova (1963) evaluated the effects of THF in mice following 2 or 6 months of exposure. Following 2 months of exposure, mice displayed symptoms of eye and respiratory tract irritation and an increase in the threshold of neuromuscular irritability. These symptoms were not reported following 6 months of exposure.

Several systemic effects have been observed following subchronic or chronic inhalation exposure to THF. Decreased body weight has been observed in rats (Horiguchi et al., 1984; Kawata and Ito, 1984). Decreased blood pressure was observed in dogs (BASF, 1938) and mice (Stasenkova and Kochetkova, 1963). Altered hematological parameters were observed in rats (NTP, 1998; Horiguchi et al., 1984), mice (NTP, 1998; Stasenkova and Kochetkova, 1963), and dogs (BASF, 1938). Rats of both sexes displayed significantly increased relative organ weights for kidney, liver, and lung, while having significantly decreased weights for thymus and spleen (NTP, 1998). Mice of both sexes showed increased relative liver weight and decreased relative spleen weight, while male mice only had decreased relative thymus weight and female mice had

increased relative lung weight (NTP, 1998). In addition, Horiguchi et al. (1984) observed increased relative weights of brain, lung, liver, pancreas, spleen, and kidney.

BASF (1938) found no histopathological effects in the heart, lungs, spleen, pancreas, or kidneys in dogs. Horiguchi et al. (1984) found no histopathological effects in brain, liver, lung, pancreas, spleen, and kidney of rats. NTP (1998) found no nonneoplastic histopathological effects in rats following both subchronic and chronic exposures, although a marginal increase in kidney adenomas and carcinomas was identified in males. However, Kawata and Ito (1984) observed hyperplasia and degeneration in the lungs and protein casts with hyaline droplet formation in the kidneys of rats exposed to THF for 12 weeks. Nonneoplastic histopathological changes observed in mice include mild hypertrophy of the muscle fibers of several organs (Stasenkova and Kochetkova, 1963) and liver centrilobular cytomegaly, uterine atrophy, and degenerative changes of adrenal cortex (NTP, 1998). Increased incidence of hepatocellular adenomas and carcinomas was also observed in female mice (NTP, 1998). The NTP (1998) study also reported a series of nonneoplastic histopathological changes in male mice in the chronic study that were attributed by the study authors to be secondary responses to inflammation in the urogenital tract. These included increased incidence of bone marrow and iliac lymph node hyperplasia, hematopoietic cell proliferation in the spleen, and thymic atrophy.

Developmental studies by the inhalation route have been conducted in both rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). In both studies and both species, maternal toxicity included symptoms of CNS effects and significant decreases in body weight accompanied by decreases in gravid uterine weight (Mast et al., 1992) or food consumption (DuPont Haskell Laboratory, 1980). Decreased fetal weight was observed at the same concentration that resulted in maternal toxicity in rats (Mast et al., 1992). In both mice (Mast et al., 1992) and rats (DuPont Haskell Laboratory, 1980), decreased fetal survival occurred at the same concentrations that resulted in maternal toxicity. With regard to potential teratogenic effects, Mast et al. (1992) noted that in mice that survived the exposure period, no increase was observed in the incidence of fetal abnormalities. However, an increased incidence of incomplete sternal ossification in rat fetuses was observed (DuPont Haskell Laboratory, 1980).

In a subchronic neurotoxicity assay (DuPont Haskell Laboratory, 1996b), the only effects observed were transient symptoms of CNS toxicity that were not observed on Monday mornings prior to the start of the weekly exposures. No permanent neurotoxic effects were observed on motor activity or in an FOB. Altered brain catecholamine levels were observed following 8 weeks of inhalation exposure (Kawata et al., 1986), and altered EEGs were observed following i.p. injection (Marcus et al., 1976). While the clinical significance of these findings is not clear in terms of assigning adverse effect levels for THF, the observation that similar brain alterations are induced by the THF metabolites GBL and GHB (NSF, 2003) suggests that these metabolites are responsible for the observed neurotoxicity of THF.

4.5.3. Mode-of-Action Information

The available data suggest that THF metabolism is extensive and that oxidative metabolism may be due to CYP450 isozymes. However, the identity of the isozymes responsible for THF metabolism has not been elucidated. In addition, whether THF or one of its metabolites is responsible for the observed toxicological effects is not known. Some mode-of-action data (BASF, 2001b) suggest that the parent compound might be the active form for liver toxicity and that metabolites might be responsible for neurological effects.

THF was evaluated in a series of short-term in vitro tests to assess its potential for cytotoxicity. The results of these studies suggest that THF is not cytotoxic.

In the two-generation reproduction study (Hellwig et al., 2002; BASF, 1996) of THF in rats by the oral route, increased kidney weights in F0/F1 adults were observed in the high-dosed groups. The mode of action for the increased kidney weight in the F0/F1 rats is unclear. Two possible modes of action were considered.

First, THF exposure by the inhalation route induces CYP450 activity in the mouse liver (Gamer et al., 2002; BASF, 2001a,b), and therefore it is possible that a similar response could occur in the rat kidney. However, data available showed that acute inhalation exposures had no effect on kidney CYP450 activity in male F344 rats (Gamer, et al., 2002; BASF, 2001a). These results are not directly comparable to the oral two-generation study since the exposure duration (acute versus subchronic) and rat strain (F344 versus Wistar) differed for the two studies. Nevertheless, the only directly available data do not support the idea that CYP450 induction is responsible for the observed increase in kidney weight. Furthermore, as discussed in detail in the context of mechanisms of liver toxicity (Section 4.4.2), since it is not known whether THF itself or a metabolite is the active moiety with respect to the kidney effects, it is not clear whether an induction of CYP450 activity is likely to increase or decrease THF toxicity in the affected organ.

Second, there is a possibility that the increase in kidney weight could be secondary to the accumulation of α_{2u}-globulin since THF induces an accumulation of α_{2u}-globulin in male rats (Gamer, et al., 2002; BASF, 2001a). However, since treatment-related increases in kidney weight were observed in females as well as males, the increase in organ weight cannot be attributed solely to this mechanism.

Decreased body weight gain in F1/F2 pups and delayed developmental stages (delayed opening of eyes and the auditory canal) in F2 pups were also observed in the high-concentration groups of the two-generation reproduction study of THF in rats by the oral route (BASF, 1996).

Two hypotheses for the observed decrease in pup body weight gain were considered. First, decreased maternal water intake during the lactation period could inhibit maternal milk production, resulting in decreased nutrition for pups and corresponding decreases in their growth, assuming that the composition of the milk did not change to maintain its nutrition value at times when water intake is low. Published correlations were not identified to determine whether the magnitude of water intake decrease in this study would have been expected to result in a

significant decrease in milk production. However, published studies do show a general correspondence between water restriction and decreased volume of milk production in both humans and livestock (Hossaini-Hilali et al., 1994; Morse et al., 1992; Dusdiecker et al., 1985; Little et al., 1980), and, therefore, the proposed explanation of decreased pup weight due to decreased milk production is biologically plausible. The temporal pattern of decreased pup body weight gain (significant decrements only during PNDs 4–14) correlates well to the postnatal lactation period where milk intake is greatest, and thus demand on a limited maternal milk supply would be expected to be most dramatic. The absence of an effect on pup body weight gain for PNDs 14–21 corresponds to the period where pups begin direct food and water intake and therefore depend less on milk production as a source of nutrition.

Whether the observed decrease in water intake was due to a toxic effect of THF or was secondary to poor palatability is not clear from the available data. No study was conducted to test specifically whether THF, at the concentrations tested, reduced water intake solely because of taste aversion. Also, the two-generation study (Hellwig et al., 2002; BASF, 1996) did not include a water-restricted control group to separate the effects of decreased water intake from those that are induced directly by THF. In some cases the temporal pattern of water intake can provide evidence for decreased palatability, where decreased water consumption at initial introduction of the treated water is greater than the decrease observed at later exposure periods. However, for the two-generation study (Hellwig et al., 2002; BASF, 1996), the decrease in water intake was not greater for week 1 versus other weeks during the premating period. This result by itself is not sufficient to determine whether decreased water intake was secondary to palatability, since water intake data for initial days of exposure were not reported (weekly summaries were provided in the report), and this is only an indirect measure of potential taste aversion.

The second hypothesis is that THF itself induces a direct effect on pup development. Several considerations provide indirect support for a role of THF in the observed decreased pup body weight gain. In the two-generation study (Hellwig et al., 2002; BASF, 1996), THF induced several developmental effects in the F2 pups (delayed opening of eyes and the auditory canal) in addition to decreased pup weight gain. While this observation that other developmental indices are affected by THF treatment supports a role of THF exposure, it could simply reflect additional developmental delays resulting from decreased milk availability. The developmental effects of THF have also been tested in inhalation exposures in rodents, which would not be subject to issues of water palatability. However, the available studies did not assess postnatal development (sacrifice was at the end of gestation) and therefore do not provide directly comparable responses to the oral two-generation study. In the inhalation studies, maternally toxic concentrations of THF reduced fetal survival and weight and increased the incidence of fetal skeletal alterations in rats and mice (Mast et al., 1992; DuPont Haskell Laboratory, 1980). These inhalation data are consistent with the hypothesis that THF can induce developmental effects. On the other hand, even though the two-generation study did not fully evaluate fetal toxicity outcomes, the absence

of a THF effect on litter size or pup weight early during the postnatal period (days 1–4) suggests that fetal effects were not occurring in the oral dosing study. One explanation for the absence of an indication of fetal effects in the two-generation study, other than dose route, is that the degree of maternal toxicity in the inhalation studies was more severe than in the drinking water study.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight of Evidence

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* following inhalation exposure to THF in humans. No human inhalation exposure studies are available for THF. However, a 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (statistically significant dose-response trend) and an increased incidence of hepatocellular adenomas and carcinomas in female B6C3F1 mice (statistically significant only at the high dose) following inhalation exposure to 200, 600, and 1800 ppm. No human or animal cancer bioassay data following oral exposure to THF are available for an assessment of human carcinogenic potential of THF. EPA's *Guidelines for Carcinogen Risk Assessment* (2005a) indicate that, for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. Based on toxicokinetic data, oral doses of THF are absorbed rapidly from the GI tract and thus would be distributed to the liver. Because the liver is a tumorigenic site in mice following inhalation exposure to THF, the delivery of THF to the liver following oral dosing suggests that it may also be tumorigenic by this route of exposure.

A full 2-year cancer bioassay by the oral route has been conducted for the THF metabolite GBL (NTP, 1992), which showed no evidence of carcinogenicity in rats (male and female) or female mice. The authors concluded that there was equivocal evidence of carcinogenic potential, based on increased incidence of adrenal medulla pheochromocytomas and hyperplasia. Mode-of-action studies for THF following exposure by the inhalation route also suggest that THF itself rather than a metabolite might be responsible for the observed liver and kidney responses. Based on these mode-of-action data and the difference in tumor responses for THF and GBL in NTP (1992) bioassays, the cancer bioassay data for THF metabolites cannot be used directly for the assessment of THF carcinogenicity in humans.

This is the first IRIS assessment for THF. Therefore, no previous characterization of cancer potential or quantitative cancer evaluation exists. THF has been tested in a standard inhalation cancer bioassay (NTP, 1998), which observed a statistically significant dose-response trend for both kidney tumors (renal tubule adenomas and carcinomas) in male rats and liver tumors (hepatocellular adenomas and carcinomas, including a statistically significant increase at the highest dose tested) in female mice. No other treatment-related increases in tumor incidence

were observed. Based on these findings, NTP (1998) concluded that there was *some evidence* of carcinogenic activity for male rats (renal tubule adenoma and carcinomas), *no evidence* of carcinogenic activity for female rats and male mice, and *clear evidence* of carcinogenic activity in female mice (hepatocellular adenoma and carcinoma).

There are some mechanistic data suggesting that the observed renal tumors in the male rats may be secondary to α_{2u}-globulin accumulation, a mode of action not relevant to humans. A U.S. EPA (1991b) guidance document outlines the following criteria for identification of an α_{2u}-globulin toxicant: (1) increased number and size of hyaline droplets in renal proximate tubule cells of treated male rats; (2) determination that the accumulating protein in the hyaline droplets is α_{2u}-globulin; and (3) presence of additional aspects of pathological sequence of lesions associated with α_{2u}-globulin nephropathy. Review of the mechanism data available for THF indicates that U.S. EPA (1991b) criteria are not met completely. A remaining area of uncertainty was the absence of detectable histopathological lesions characteristic of this mode of action (BASF, 2001a; NTP, 1998). Furthermore, absence of evaluation of α_{2u}-globulin accumulation and potential precursor events (e.g., cell proliferation) in female rats hinders the determination of the response specificity. The kidney tumors observed in the cancer bioassay in male rats may be considered relevant to the assessment of the carcinogenic potential of THF to humans.

However, the evidence of the male rat kidney tumors is weak. The observed renal tumors may be secondary to α_{2u}-globulin accumulation, but, because of the absence of detectable histopathological lesions characteristic of this mode of action, the result might reflect the weak potency of THF to induce α_{2u}-globulin, which would be consistent with the detection of α_{2u}-globulin accumulation only when sensitive detection methods were used and may explain in part the weak tumorigenic response observed in the chronic bioassay (NTP, 1998). According to U.S. EPA (1991b), the presence of pathological lesions is a required element for concluding that α_{2u}-globulin accumulation is the mode of action for kidney tumors. Therefore, in the absence of these pathological findings, the male rat tumors should be presumed relevant for the cancer risk assessment of THF. It is noteworthy, however, that the technical report recommendations (U.S. EPA, 1991b) were based largely on patterns of effects observed for more potent inducers of α_{2u}-globulin accumulation than THF appears to be and may not account for subtle effects caused by less potent inducers of this mechanism. Therefore, it remains uncertain whether THF may act through the accumulation of α_{2u}-globulin accumulation.

It is plausible that advanced chronic progressive nephropathy (CPN) may play a role in the incidence of atypical tubule hyperplasia (ATH) and perhaps THF-induced kidney tumors in male rat kidneys (see Appendix A). CPN is an age-related renal disease of laboratory rodents that occurs spontaneously in high incidence. There was no difference in the incidence or severity of CPN in male rats of the NTP 2-year carcinogenicity study on THF (both the control and high-

dose groups had the same incidence of end-stage renal CPN). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue. No data in the peer-reviewed literature are available that support a role of CPN in the induction of THF-induced kidney tumors in male rats. The exact mode of action of THF for the male rat kidney tumors is still unclear. The kidney tumors observed in the cancer bioassay in male rats were considered relevant to the assessment of the carcinogenic potential of THF to humans.

For the liver tumors in mice, some mechanistic data suggest that THF may induce cell proliferation and lead to a promotion in the growth of preinitiated cells. However, key precursor events linked to observed cell proliferation have not been clearly identified. Although increased cell proliferation was noted in short-term studies, the data are not adequate to support the hypothesized mode of action. The absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study suggests that cell proliferation might not be a sustained response even with continued dosing. Therefore, while the cell proliferation event meets the requirement of showing the expected temporal relationship at early time points, it is not clear that the effect is sustained for a sufficient duration to adequately explain the late onset of tumors. Furthermore, key precursor events linked to observed cell proliferation have not been clearly identified. The data on other modes of action are too limited to establish the mode of action for the liver tumor induction of THF. Therefore, the mode of action for liver tumor induction by THF is still unknown.

Overall, although a number of possible modes of action for the carcinogenicity of THF have been proposed, there are inadequate data to support any conclusive mode of carcinogenic action.

The weight of evidence suggests that THF is carcinogenic for more than one species, sex, and site; therefore, there is suggestive evidence of carcinogenic potential following exposure to THF in humans (U.S. EPA, 2005). This descriptor is consistent with the NTP (1998) weight-of-evidence evaluation. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of THF is deemed appropriate.

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

As discussed in Section 4.1, there are no human studies investigating the carcinogenic effects of THF following inhalation exposure. However, the NTP (1998) chronic inhalation exposure bioassay in laboratory animals was adequately designed to assess the carcinogenic potential of lifetime inhalation exposure to THF. This study involved exposure of F344/N rats (50/sex/group) and B6C3F1 mice (50/sex/group) to 0, 200, 600, and 1800 ppm (0, 590, 1770, and 5310 mg/m^3) THF for 6 hours/day, 5 days/week for 105 weeks. The study provided *some evidence* for THF carcinogenicity in male rats (renal tubular adenomas and carcinomas) and *clear evidence* of carcinogenicity in female mice (hepatocellular adenomas and carcinomas).

For the male rats, a statistically significant treatment-related trend was observed for combined incidences of renal tubular epithelial adenomas or carcinomas (1/50, 1/50, 4/50, and 5/50) (NTP, 1998). The response was predominantly benign except for two carcinomas present at the high dose. The individual dose incidences of these combined kidney adenomas and carcinomas in the male rats appeared to exceeded the incidence of these tumors in F344/N historical controls (rate: 0.9 ± 1.3%; range: 0–4%) but were not statistically significant (NTP, 1998).

In female mice there was a statistically significant increased incidence of hepatocellular neoplasms (combined adenomas or carcinomas) at the high dose (1800 ppm) and a positive trend for these hepatocellular neoplasms following exposure to 200 and 600 ppm THF compared with controls (17/50, 24/50, 26/50, and 41/48) (NTP, 1998). The females also showed an increased incidence in hepatocellular carcinomas (not significant; 6/50, 10/50, 10/50, and 16/48). There was not a statistically significant increased incidence of hepatocellular adenomas or carcinomas in male mice (35/50, 31/50, 30/50, and 18/50).

As discussed in Section 4.4.3, results from genotoxicity studies for THF are mostly negative and provide limited evidence to suggest a genotoxic mode of action. All bacterial mutation assays were negative for THF genotoxicity. In vitro genotoxicity assays with eukaryotic cells also proved to be negative with the exception of a slight increase in chromosomal aberrations in Chinese hamster ovary cells with metabolic activation (Galloway et al., 1987). In vivo studies support the assertion that THF is nongenotoxic but did not evaluate possible genotoxicity in target tissues. Only one study (NTP, 1998) provided equivocal positive evidence of clastogenicity in a mouse erythrocyte micronucleus assay (only in the mid-concentration males).

4.6.3. Mode-of-Action Information

Both hepatocellular and renal adenomas and carcinomas are observed following inhalation exposure to THF (NTP, 1998). However, the mode of carcinogenic action of THF is unclear. THF is not genotoxic, as the results of the mutagenicity tests conducted by NTP (1998) provide little evidence of mutagenic activity, with most data determined to be conclusively negative (Section 4.4.3). There are mode-of-action data suggesting that the induction of kidney tumors in male rats and liver tumors in female mice may involve the accumulation of α_{2u}-globulin in the kidney and increased cell proliferation in the liver, respectively.

4.6.3.1. *Kidney Tumors*

Description of the hypothesized mode of action

Summary description of the hypothesized mode of action

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, a number of chemicals have been shown to induce accumulation of α_{2u}-globulin in hyaline droplets in male rat kidney. The α_{2u}-globulin accumulation in hyaline droplets initiates a sequence of events that leads to renal nephropathy and, eventually, to renal tubular tumor formation. The phenomenon is unique to the male rats since female rats and other laboratory mammals administered the same chemicals do not accumulate α_{2u}-globulin in the kidney and do not develop renal tubule tumors (U.S. EPA, 1991b).

Identification of key events

For chemicals inducing kidney tumors in male rats involving the α_{2u}-globulin accumulation mode of action, the following events occur after binding of the chemicals or their metabolites specifically, but reversibly, to α_{2u}-globulin:

- Increased number and size of hyaline droplets in renal proximal tubule cells of treated male rats
- Accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules due to the resistance of the α_{2u}-globulin chemical complex to hydrolytic degradation by lysosomal enzymes
- Induction of typical pathological lesions associated with α_{2u}-globulin nephropathy (e.g., single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia).

Discussion of the experimental support for the hypothesized mode of action

Strength, consistency, specificity of association

Chhabra et al. (1998) published a summary of the NTP (1998) bioassay in which these authors presented data on the accumulation of α_{2u}-globulin (as indicated by protein droplets) in male rat kidney following 13 weeks of exposure to 1800 ppm THF. The authors observed qualitative differences in the appearance of protein droplets of the kidneys of control versus male rats exposed to 1800 ppm THF. Differences in the appearance and location of protein droplets in the male rat kidneys for control and high-concentration group rats were noted. Protein droplets were described as finer and more densely and diffusely distributed in tubular epithelial cells in the outer cortex for control rats. In the high-concentration rats, protein droplets were characterized as coarser and concentrated in scattered foci in the outer cortex. However, the

average severity grades for the accumulation of protein droplets did not differ and no other differences in the incidence of nonneoplastic lesions in the male rat kidneys were observed. Therefore, no clear signs of treatment-related pathological lesions in the kidney were found in the NTP (1998) study.

BASF (1998) reevaluated kidney tissues of male rats to examine the relationship between cell proliferation responses and increase in kidney tumors observed following THF administration in the NTP (1998) study. Histopathological examination and evaluation of cell proliferation as measured by PCNA staining was conducted for tissue samples from the 0, 200, 600, and 1800 ppm (0, 590, 1770, and 5310 mg/m^3) exposure groups (10/group) from the NTP (1998) subchronic (13 weeks) study. Kidney tissues from the cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and the inner medulla were evaluated separately. The histopathological examination revealed increased incidence of moderate grade hyaline droplet accumulation in the male rat kidney tissues of the high-concentration group as compared with controls, but these changes were not accompanied by evidence of cell degeneration. No increase in cell proliferation was found in any of the individual kidney compartments or in evaluation of all compartments combined. Cell proliferation index was statistically significantly decreased in individual kidney compartments, although these changes did not show a concentration-dependent pattern. No other differences among controls and exposure groups were noted.

Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) conducted a series of mode-of-action studies in the kidneys of male F344 rats (six/group) where THF-treated animals developed tumors. The endpoints that were evaluated included α_{2u}-globulin accumulation, cell proliferation, and apoptosis. Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed nose-only to average THF concentrations of 0, 598, 1811, or 5382 mg/m^3 (0, 199, 604, or 1794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the kidney. Additional evaluations in these same organs included measurements of cell proliferation (S-phase response by BrdU staining) and TUNEL apoptosis assay.

Results of the study (Gamer et al., 2002; BASF, 2001a) provide some evidence for α_{2u}-globulin accumulation as a plausible mode of action of THF-induced kidney carcinogenicity. Specifically, THF exposure induced α_{2u}-globulin accumulation in male rats treated in a dose-related manner (see Table 4-8) after 5- or 20-day exposures (6 hours/day). The accumulation of α_{2u}-globulin as measured by immunohistochemistry was supported by histopathological evaluation of hyaline droplets in the kidneys of control and high-concentration animals exposed to THF for 20 days. The incidence of proximal tubule cells with grade 2 (slightly increased)

staining for hyaline droplets (putatively α_{2u}-globulin) was 5/6 for exposed animals versus 1/6 for controls.

The study also showed that focal areas of α_{2u}-globulin accumulation corresponded to areas of increased cell proliferation. Although no significant increase in labeling index in the renal cortex was determined by standard assessment methods, focal areas of increased BrdU labeling were noted. Quantitation of these areas revealed increased cell proliferation in subcapsular proximal tubules (cortex 1) in animals exposed to THF at the mid and high concentrations for 20 days and at the high concentration for 5 consecutive days. No increase in labeling was observed in the groups given a 21-day recovery period. An increase in cell proliferation was also noted in the proximal tubules between the outer stripe of the outer medulla and the subcapsular layer (cortex 2) at the highest concentration following 20 exposures. The number of cells undergoing apoptosis was significantly increased in the high-concentration groups exposed for 5 days and observed for 21 days or after 20 exposure days. Marginal increases were observed in the mid-concentration groups for these two exposure regimens, but the results were not statistically significant (see Table 4-8).

In a Japanese study, Kawata and Ito (1984) reported protein casts and hyaline droplets in the kidneys of THF-exposed male Wistar rats. No other details of the study are available.

No evaluation of α_{2u}-globulin accumulation was reported on the female rats treated with THF. Absence of mode-of-action data in female rats hinders the determination of the response specificity.

Dose-response concordance

THF exposure induced α_{2u}-globulin accumulation in male rats treated under all three exposure regimens in the study by Gamer and coworkers (Gamer et al., 2002; BASF, 2001a). Increases were generally concentration related, with increases at the high concentration ranging from 175 to 280% of control levels for cortex 1 and from 188 to 324% of control levels for cortex 2, among the three exposure regimens. When the whole cortex was used as the labeled area for the analysis, accumulation was significantly elevated beginning at the low concentration and following 5 consecutive days or 20 days of exposure. Maximum effects observed at the high concentration ranged from 178 to 299% of controls among the three exposure regimens. Increased cell proliferation and apoptosis in kidney of animals exposed to THF for 20 days also appeared to show a dose-response relationship (see Table 4-8).

Temporal relationship

The mode-of-action data were obtained from short-term exposures (5 or 20 days) of THF. Except for some qualitative differences in the appearance of protein droplets of the kidneys of control versus male rats exposed to 1800 ppm THF, no clear signs of treatment-related

pathological lesions in the kidney were found in the 2-year bioassay of NTP (1998). No increase in cell proliferation was found in any of the kidney compartments in the 13-week study of BASF (1998). Therefore, a temporal relationship of the key events to male rat kidney tumor induction cannot be established.

Biological plausibility and coherence

The concordance between α_{2u}-globulin accumulation, cell proliferation, and induction of apoptosis in the renal cortex, with doses that induce kidney tumors in cancer bioassays, appears to support the involvement of these mechanisms in THF-induced rat kidney tumors. However, no increase in renal tubule hyperplasia or mineralization was observed in the NTP (1998) study. The detection of α_{2u}-globulin accumulation only when sensitive detection methods were used (i.e., immunohistochemical staining as opposed to standard staining for histopathological examination) suggests that the responses are weak (Chhabra et al., 1998; NTP, 1998). Furthermore, the observed cell proliferation response, which was increased to a maximum of 298% of controls when selected for focal areas of proliferation, was minimal as compared with cell proliferation responses induced by other well-characterized inducers of α_{2u}-globulin accumulation (Gamer et al., 2002; BASF, 2001a; U.S. EPA, 1991b). There is also an uncertainty regarding the specificity of the relationship between cell proliferation (a putative tumor precursor event) and the observed α_{2u}-globulin accumulation, since the mode-of-action study by Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) did not include a similar analysis of cell proliferation in female rat kidneys in which α_{2u}-globulin accumulation does not occur. A major area of uncertainty arises from the absence of detectable histopathological lesions characteristic of this mode of action. No treatment-related renal histopathology or hyaline or granular casts were noted in the BASF study (Gamer, et al., 2002; BASF, 2001a). According to U.S. EPA (1991b), the presence of pathological lesions is one of the required elements for concluding that α_{2u}-globulin accumulation is the mode of action for kidney tumors. Because of the weak response in α_{2u}-globulin accumulation and cell proliferation and the absence of the detectable pathological findings, the evidence for this mode of action is equivocal.

Consideration of the possibility of other modes of action

It is plausible that advanced CPN may play a role in the incidence of ATH and perhaps THF-induced kidney tumors in male rat kidneys (see Appendix A). CPN is an age-related renal disease of laboratory rodents that occurs spontaneously with high incidence. There was no difference in the incidence or severity of CPN in male rats of the NTP (1998) 2-year carcinogenicity study on THF (both the control and high-dose groups had the same incidence of end-stage renal CPN). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue.

No data in the peer-reviewed literature are available that support a role of CPN in the induction of THF-induced kidney tumors in male rats. The exact mode of action of THF for the male rat kidney tumors is still unclear.

Conclusions about the hypothesized modes of action

Generally, kidney tumors observed in cancer bioassays are assumed to be relevant for assessment of human carcinogenic potential. However, for male rat kidney tumors, when the mode-of-action evidence convincingly demonstrates that the response is secondary to α_{2u}-globulin accumulation the tumor data are not used in the cancer assessment (U.S. EPA, 1991b). There are some data suggesting that male rat kidney tumors, following the inhalation exposure observed in the NTP (1998) bioassay, may be due to the accumulation of α_{2u}-globulin. The criteria for demonstrating this mode of action for risk assessment purposes have been clearly defined (U.S. EPA, 1991b). Three core criteria must be met: (1) increase in hyaline droplets in the renal proximal tubule cells; (2) determination that the accumulating protein in the droplets is α_{2u}-globulin; and (3) presence of additional pathological lesions associated with α_{2u}-globulin. Review of the mode-of-action data of THF indicates that criteria (1) and (2) are met but criterion (3) is not. A remaining area of uncertainty was the absence of detectable histopathological lesions characteristic of this mode of action (BASF, 2001a; NTP, 1998). Furthermore, absence of evaluation of α_{2u}-globulin accumulation and potential precursor events (e.g., cell proliferation) in female rats hinders the determination of the response specificity. However, an increased incidence of kidney tumors was not observed in female rats in the NTP (1998) study.

4.6.3.2. *Liver Tumors*

Description of the hypothesized mode of action

Summary description of the hypothesized mode of action

Induction of a cell proliferation response in the liver by chemicals is one biologically plausible mode of action for the liver tumorigenesis in rodents. Sustained increase in cell proliferation may lead to the promotion of growth of preinitiated cells and subsequently to tumorigenesis.

Identification of key events

There are many mechanisms by which chemicals may induce a mitogenic response. For instance, increased cell proliferation in the liver may be induced by cytotoxic chemicals subsequent to regenerative growth of the injured tissue and/or cell death. Changes in cellular apoptosis rates can also impact the net rate of tissue growth. Key events for this mode of action may include histopathological evidence of cytotoxicity/necrosis, regenerative growth, and/or apoptosis.

Discussion of the experimental support for the hypothesized mode of action

Strength, consistency, specificity of association

BASF (1998) evaluated the liver tissues from female mice from the NTP (1998) study to examine the relationship between cell proliferation responses and increase in tumors observed in these tissues following THF administration. Histopathological examination and evaluation of cell proliferation as measured by PCNA staining was conducted for tissue samples from the 0, 200, 600, and 1800 ppm (0, 590, 1770, and 5310 mg/m^3) exposure groups (10/group) from the NTP (1998) subchronic (13 weeks) study. No treatment-related histopathology was observed in the female mouse liver tissues. The cell proliferation index was mildly increased in tissues from the high-concentration mice by 39% over controls. However, this result was not statistically significant and was noted as being predominately based on the results from 2 of 10 animals. Furthermore, no clear concentration-response pattern was observed, and a significant decrease in proliferation index was observed in the mid-concentration group. Based on these results, the study authors concluded that the examination of the tissues from the 13-week NTP (1998) study revealed no clear increase in cell replication that can be correlated to a tumorigenic mechanism. Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) evaluated a series of endpoints in female B6C3F1 mice (10/group plus 5 in the control and high-concentration enzyme assays) in liver tissues for which THF-treated animals develop tumors. Animals were placed in one of three groups that were exposed 6 hours/day for either 5 consecutive days, 5 consecutive days followed by a 21-day observation period, or 20 consecutive days over a period of approximately 28 days. Test animals were exposed nose only to average THF concentrations of 0, 598, 1811, or 5382 mg/m^3 (0, 199, 604, or 1794 ppm), corresponding to the concentrations used in the NTP (1998) cancer bioassay. Concentrations adjusted for continuous exposure were 0, 107, 323, or 961 mg/m^3. For the animals in each of the four concentration groups, a full necropsy was done, including histopathological evaluation of the liver. Additional evaluations in these same organs included measurements of cell proliferation (S-phase response by BrdU staining) and TUNEL apoptosis assay. Since chemical exposures can have varying affects in different regions of the liver lobule, cell proliferation was evaluated separately for zone 1 (the region adjacent to the portal triad), zone 3 (the region adjacent to the central vein), and zone 2 (the area of the lobule intermediate between zones 1 and 3).

THF exposure appeared to induce cell proliferation (see Table 4-9) in the female mouse liver. Increased cell proliferation was observed in zones 2 and 3 of the liver of the high-dose mice following THF exposure for 5 days and in zone 3 following 20 exposures. Coincident with the increase in BrdU labeling, the mitotic index was increased in zone 3 after 5 or 20 exposures in the high-concentration groups. No concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period, suggesting that the increases in cell proliferation may be an adaptive effect. No treatment-related change in the number of liver cells undergoing apoptosis was observed.

Dose-response concordance

Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) reported increased cell proliferation following short-term inhalation exposures at concentrations corresponding to those that were tumorigenic in the NTP (1998) bioassay. Therefore, this event appeared consistent with the expected dose response as compared to the tumor outcome.

Temporal relationship

Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) reported increased cell proliferation in the liver of the high-dose female mice following short-term inhalation exposures (5 or 20 days) of THF. However, no concentration-dependent increase in BrdU labeling was observed in the animals given a 21-day recovery period, suggesting that the increases in cell proliferation may be an adaptive effect.

Biological plausibility and coherence

Although increased cell proliferation was noted in short-term mode-of-action studies, the data are not adequate to identify key events that precede this effect. In the earlier of these two mode-of-action studies (Gamer et al., 2002; BASF, 2001a) it was not entirely clear if the lower degree of BrdU staining after 20 exposures as compared to 5 exposures (see Table 4-8) represented fluctuation around an average increase in cell proliferation or a decrease in the rate of proliferation with continued exposure. While the observation that the mitotic index did not similarly decrease after 20 exposures supports the former conclusion, the absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study as reported by BASF (1998) suggests that cell proliferation might not be a sustained response even with continued dosing. Therefore, while the cell proliferation event meets the requirement of showing the expected temporal relationship at early time points, it is not clear that the effect is sustained for a sufficient duration to adequately explain the late onset of tumors.

There are many mechanisms by which THF might induce a mitogenic response; however, the available data are not sufficient to determine specific mechanisms likely to be involved. In the NTP (1998) bioassay, no clear concentration-dependent increase in necrosis was observed, although the incidence of necrosis was slightly elevated at the high concentration. Gamer and coworkers (Gamer et al., 2002; BASF, 2001a) reported no histopathological evidence of cell degeneration at concentrations that induced cell proliferation. Other in vitro studies did not suggest that THF is cytotoxic (Matthews et al., 1993; Dierickx, 1989; Curvall et al., 1984). Taken together, these data suggest that THF-induced cell proliferation is not secondary to regenerative hyperplasia. Changes in cellular apoptosis rates can also impact the net rate of tissue growth. However, the single study that evaluated this endpoint (Gamer et al., 2002; BASF, 2001a) suggested that THF exposure has little impact on apoptosis in the livers of female

mice. Taken together, key precursor events linked to observed cell proliferation have not been clearly identified for THF.

Consideration of the possibility of other modes of action

One possible mode of carcinogenic action of THF is the ability of THF to inhibit GJIC. In a study by Chen et al. (1984), cocultures of 6-thioguanine-sensitive and resistant Chinese hamster V79 fibroblast cells were treated with THF, and the degree of metabolic cooperation was determined by the survival of the resistant cells. The killing of resistant cells serves as an indicator of metabolic cooperation because the toxic 6-thioguanine metabolite that is formed only in the sensitive cells can be passed on to normally resistant cells when gap junctions are intact. Therefore, robust growth of the resistant cells in this assay system would suggest that GJIC is inhibited. THF was judged to be positive (as defined by at least a doubling in recovery of resistant colonies) in the metabolic cooperation assays, suggesting that THF can inhibit GJIC. The recovery rate of resistant cells increased with increasing concentration (up to 100 μL of THF/5 mL of medium). Although there appears to be a correlation between inhibition of GJIC and mouse liver carcinogenesis by some nongenotoxic carcinogens, the mechanism is unclear (Klaunig et al., 1998). The data on gap junction intercellular communication presented by Chen et al. (1984) are too limited to establish that this is the mode of action for the liver tumor induction of THF.

As the major metabolite of THF, GHB, can be converted to GABA, it has been hypothesized that the production of GABA from THF may perturb the cellular level of putrescine (1,4-diaminobutane), since putrescine is the primary source of GABA in many tissues. Putrescine is required for proper functioning of the cell cycle and for cell growth (Lopez et al., 1999) and has been shown to induce cell transformation and stimulate the expression of *c-fos*, a proto-oncogene (Tabib and Bachrach, 1999). Therefore, it was postulated that THF exposure would increase tissue levels of GABA and putrescine, which in turn might promote cell growth and carcinogenesis. However, the link between GABA and putrescine has not been thoroughly investigated. While this mode of action provides a biologically plausible basis for THF-induced cell proliferation and subsequent carcinogenesis, it has not been tested directly for THF.

Conclusions

Although increased cell proliferation was noted in short-term studies, the data are not adequate to support the hypothesized mode of action. The absence of a significant increase in cell proliferation in tissues obtained from the subchronic NTP (1998) study suggests that cell proliferation might not be a sustained response even with continued dosing. Therefore, while the cell proliferation event meets the requirement of showing the expected temporal relationship at early time points, it is not clear that the effect is sustained for a sufficient duration to adequately

explain the late onset of tumors. Furthermore, key precursor events linked to observed cell proliferation have not been clearly identified. The data on other modes of action are too limited to establish the mode of action for the liver tumor induction of THF. Therefore, the mode of action for liver tumor induction by THF is still unknown.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

No adequate studies on the potential reproductive or developmental toxicity of THF in humans were available. However, these endpoints have been evaluated following oral and inhalation exposures to THF in animal studies and oral studies with THF metabolites. A one-generation screening assay (BASF, 1994) and a more comprehensive two-generation assay (BASF, 1996) were conducted for THF administered in drinking water of rats. Decreased body weights in both male and female pups and delayed eye and auditory canal opening in male pups were observed. There are no data that indicate why developmental delays in eye and auditory canal opening are observed in male pups but not female pups. These developmental effects were observed at doses that also induced maternal effects (although the maternal effects were only of minimal severity). For the THF metabolite GBL, no maternal or developmental effects were observed in rats (Kronevi et al., 1988); since no effects were observed, this study is not informative in comparing relative susceptibility of adult and young animals. Decreased testicular weight was reported in a short-term reproductive study for GBL (Debeljuk et al., 1983), but no impairment of fertility was reported in the oral two-generation study for THF (BASF, 1996). Developmental studies by the inhalation route have been conducted in both rats (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). Mast et al. (1992) reported decreased fetal survival and incidence of sternal ossification in mice and decreased fetal body weight in rats. DuPont Haskell Laboratory (1980) reported decreased fetal weight and skeletal alterations. In these inhalation studies, developmental effects were only observed at concentrations that also induced maternal toxicity.

Comparisons of maternal to developmental effect levels can be useful for evaluating the susceptibility of young animals. The inhalation data for THF suggest that fetuses are not likely to be more susceptible than adult animals. This conclusion is supported by the observation that in the inhalation toxicity database (see Table 4-12) the LOAELs for systemic toxicity in adult animals are significantly lower than the LOAELs for developmental toxicity. However, the inhalation developmental studies are limited, since they did not provide an evaluation of postnatal development. In the only available multigeneration study for THF, postnatal development (decreased pup body weight gain and delayed eye and auditory canal opening) was affected at drinking water concentrations that had minimal effects on the dams. The results from the two-generation study indicate that the early postnatal period is a period of increased susceptibility, but this conclusion is very uncertain since the changes in pup body weight may be

explained by effects on maternal water intake. Furthermore, the related measure of fetal weight was not affected in the inhalation studies or in the oral developmental study for GBL.

Only one study was identified that specifically evaluated the effect of age on toxicity of THF. Kimura et al. (1971) estimated oral LD_{50} values for a variety of solvents, including THF, for newborn, 14-day-old, young adult, and older adult rats. The oral LD_{50} values for THF were estimated as 2.3 mL/kg for 14-day-old rats, 3.6 mL/kg for young adult rats, and 3.2 mL/kg for older adult rats; none of these values were statistically different. However, the authors report that the newborn animals were much more susceptible than the other age groups, in which doses of 1 mL/kg of all the solvents tested, including THF, were generally fatal. Since sensitivity was increased in newborns for all the solvents tested, it is not clear whether the increased sensitivity to THF was due to its inherent toxicity to newborn rats or whether some other aspect of the study protocol was responsible. The study results suggest that young animals are at best marginally more susceptible to oral THF exposure than adult animals to high-dose effects.

No toxicokinetic data are available to evaluate potential childhood susceptibility. As a result, the role of age-dependent differences in THF metabolism could not be evaluated.

The overall data are not sufficient to conclude with certainty whether children are likely to be more susceptible to THF toxicity than adults. Adequate studies directly testing the systemic effects of THF in animals of different ages, as well as data on relevant metabolic parameters, are lacking. However, the occurrence of developmental toxicity only at maternally toxic doses suggests that children may not be more susceptible to THF than adults.

4.7.2. Possible Gender Differences

No adequate human studies on gender-based differences in THF toxicity are available. Several toxicity studies of acute, subchronic, or chronic duration in animals have evaluated the toxicity of THF in both males and females administered similar doses. In general, a similar spectrum of noncancer endpoints and effect levels has been observed in both sexes for oral (BASF, 1996; Komsta et al., 1988) and inhalation (NTP, 1998; DuPont Haskell Laboratory, 1996b) exposure studies. However, in the NTP (1998) subchronic study, uterine histopathology changes were observed in mice, but no histopathological effects on the uterus were noted in the companion chronic bioassay (NTP, 1998) or in a short-term inhalation study that evaluated histopathology of the uterus (BASF, 2001a). Changes in uterine weight (not statistically significant) were reported in the short-term study (BASF, 2001a). None of the available studies that evaluated reproductive capacity (BASF, 1996, 1994) suggested that either male or female fertility are targets for THF toxicity.

In addition, a comprehensive toxicokinetics study of THF following oral dosing of rats and mice of both sexes was conducted by DuPont Haskell Laboratory (1998). The AUC was higher in males, and the corresponding clearance of THF-associated radioactivity from the blood was lower in males of both species. This result might suggest that there are gender differences in

THF metabolism, since absorption and distribution of THF were similar for males and females. The available data suggest that THF metabolism is extensive and that oxidative metabolism is due to CYP450 isozymes. However, the identities of the isozymes responsible for THF metabolism have not been elucidated. In vitro evidence suggests that there are species differences in THF metabolism (DuPont Haskell Laboratory, 2000), and, therefore, the differences in THF metabolism between male and female rodents cannot be used to infer the relationship in THF metabolism between sexes in humans. As noted above, whether THF or one of its metabolites is responsible for each of the observed toxic effects has not been clearly demonstrated. As a result of these considerations, the implications of sex-based differences in metabolism cannot be determined.

A significant gender difference in response observed following exposure to THF is the sex-specific induction of kidney tumors in male rats and liver tumors in female mice (see Section 4.6.2), although the absence of an effect in male mice may be due to the apparently higher susceptibility to narcosis (and resulting mortality) in male mice in the chronic inhalation bioassay (NTP, 1998).

The overall similarity in noncancer toxicity between male and female rodents in a variety of bioassays and the absence of functional effects on male or female fertility suggest that gender-based differences in susceptibility to THF are likely to be limited. However, a number of findings raise questions about the potential for increased susceptibility based on gender, including potential effects in the uterus of mice, apparent sex-specific tumor formation, and toxicokinetic differences between male and female rodents.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Based on the currently available subchronic and chronic studies (see Sections 4.2 and 4.3), the oral database is minimally sufficient to adequately characterize the hazard posed by THF. The 4-week oral drinking water study by Komsta et al. (1988) in rats is inadequate to derive an RfD because of the short duration and failure to perform histopathology on the lower dose groups. The one-generation reproductive toxicity (dose range-finding) study (BASF, 1994) and two-generation reproductive toxicity study (Hellwig et al., 2002; BASF, 1996) in rats (both in drinking water) included exposures for approximately 90 days, but the one-generation study did not include any histopathological examination of tissues and the two-generation study did not include the comprehensive examination of all organs and systems that would be done in a well-conducted subchronic bioassay. The two-generation study (Hellwig et al., 2002; BASF, 1996) provided the results of histopathologic examinations of the liver, kidney, digestive, and reproductive organs in male and female rats. Both the one-generation (BASF, 1994) and the two-generation (Hellwig et al., 2002; BASF, 1996) studies identified increased kidney weight and decreased pup body weight gain as the two most sensitive effects. Although both studies identify approximately equivalent NOAEL/LOAEL boundaries, the two-generation study is most appropriate as the principal study because it used a narrower range of exposure concentrations and is the more comprehensive of the two studies. The one-generation study is considered as supporting the results of the two-generation study.

Based on the limitations in the oral database for THF, alternative approaches for deriving the chronic RfD were considered: (1) use the two-generation study as the basis for an RfD as supplemented by the inhalation data for THF and the oral data for THF metabolites, (2) derive an RfD from the inhalation data by using a route-to-route extrapolation approach, or (3) derive an RfD for THF based on the available data from its metabolites. The first option (derive an RfD based on the two-generation study) was selected based on the considerations discussed below.

With regard to using the inhalation data to support the chronic RfD or directly as the basis for identifying the principal study, the data would need to address portal-of-entry effects, support the idea that the systemic effects relevant to oral exposure would also have been detected following THF exposure by the inhalation route, and provide a robust understanding of route-dependent THF kinetics. THF exposure by the inhalation route induces respiratory tract effects consistent with irritation, and therefore these effects are likely to represent portal-of-entry toxicity (Kawata and Ito, 1984). Portal-of-entry effects following exposure by the inhalation route are not useful for supplementing the oral toxicity database. However, the subchronic NTP (1998) study in rats identified forestomach histopathology changes in the high concentration

group that were attributed to ingestion of THF in the inhalation study. This result would suggest that portal-of-entry effects might also be important for exposure by the oral route. Since the BASF oral dosing study (Hellwig et al., 2002; BASF, 1996) did include an evaluation of the digestive tract, this aspect of the database was adequately evaluated.

Comparing the array of responses observed in the subchronic inhalation studies versus the subchronic oral studies, suggests that the inhalation data do not completely represent the potential toxicity of THF following oral dosing. Therefore, deriving an RfD based on the available inhalation study is not appropriate. The clearest systemic toxic effect induced by THF inhalation was clinical signs of CNS toxicity (narcosis), which were observed in both rats and mice in studies of subchronic exposure duration (NTP, 1998; DuPont Haskell Laboratory, 1996b). Liver and thymus weight changes are other potential susceptible responses observed following longer-term inhalation exposures in mice (NTP, 1998), although the degree to which the observed effects at low concentrations are of toxicological relevance is not clear. The subchronic NTP (1998) study demonstrated that B6C3F1 mice were more susceptible than F344 rats to the toxicity of THF. On the other hand, a similar NOAEL/LOAEL boundary was identified for CNS effects in a subchronic neurotoxicity assay in Crl:CD Br rats (DuPont Haskell Laboratory, 1996b) as was identified in the B6C3F1 mice in the NTP (1998) study. Other effects observed in subchronic inhalation studies were decreased body weight (Horiguchi et al., 1984; Kawata and Ito, 1984), decreased blood pressure (NTP, 1998; Stasenkova and Kochetkova, 1963; BASF, 1938), altered hematological parameters (Horiguchi et al., 1984; Stasenkova and Kochetkova, 1963), and a variety of organ weight changes (NTP, 1998; Horiguchi et al., 1984). In contrast to the inhalation data, the oral two-generation study (Hellwig et al., 2002; BASF, 1996) identified decreased body weights in both male and female pups and delayed eye and auditory canal opening in male pups as the most susceptible effects, with minimal effects on body weight and increased kidney weight observed in adult animals.

A second complicating factor in using the inhalation data is the absence of data on differences in the toxicokinetics of oral versus inhaled doses. This data gap complicates the determination as to whether reliance on inhalation data to identify critical target organs following oral dosing is appropriate. THF absorption by the oral route is expected to be almost complete (never more than 1.5% of THF recovered in the feces of either rats or mice). Similar to oral exposure, THF is clearly well absorbed by the inhalation route in humans, based on measured uptake rates (Kageyama, 1988; Wagner, 1974) or the rapid appearance of THF in biological media (Droz et al., 1999). Observations in animal inhalation studies of THF tissue distribution also support this conclusion (Kawata and Ito, 1984). While there are studies by both the oral (DuPont Haskell Laboratory, 1998) and inhalation (Elovaara et al., 1984; Kawata and Ito, 1984) routes that evaluate the distribution of THF, they are not useful for comparing the similarities of THF distribution after oral and inhalation exposure. First, the studies did not all evaluate distribution by using the same duration of exposure, the same species, or the same spectrum of

tissues. Second, the studies used different approaches to measuring THF concentration in tissues. In the oral study, THF concentration was measured as ^{14}C activity, which could have represented both parent compound and metabolites. In the inhalation studies, concentration of THF as the parent compound was measured. Thus, the results obtained in these studies are not directly comparable. The comparison of systemic toxicity across routes is further hampered by the likely in vivo metabolism of THF in the liver (BASF, 2001a,b; DuPont Haskell Laboratory, 2000, 1998), which would suggest that first-pass metabolism would play a significant role in route-specific toxicity. Since it is not known whether the parent chemical or a metabolite is responsible for each of the observed target organ effects, it cannot be determined whether systemic targets of toxicity following oral dosing would be enhanced or decreased relative to inhalation exposures.

A third alternative that was considered was to derive the RfD for THF based on the oral toxicity data for the THF metabolites GBL and GHB. However, there are a number of uncertainties in applying this approach for the derivation of the RfD for THF that result in lower confidence in the RfD. Specifically, there is not a sufficient understanding of the role of the parent compound and/or metabolites in THF-induced toxicity. It is conceivable that the RfD could be based on metabolite information when there are no adequate data for the parent compound. It is also plausible to use metabolite data when the metabolite is shown to be the active form of the parent compound that induces the critical effect. The kinetics of metabolism are assumed to be sufficiently understood in order to back calculate the administered dose of parent compound from the target tissue dose of the active metabolite. Neither of these two circumstances applies to THF, and in this case the data suggest that the metabolite data are not appropriate as the basis for deriving the chronic RfD for THF as discussed below.

A basic requirement for using the data on metabolites in a quantitative fashion for the dose-response assessment is a demonstration that the critical effects following THF administration can be attributed to the toxicity of metabolites. Table 5-1 provides a qualitative comparison of identified target organs of THF, GHB and GBL. This comparison is qualitative in nature, since the absence of identified effects might reflect limitations in the design of available studies (i.e., differences in route of exposure and dose) rather than the absence of biological potency. Nevertheless, these data suggest that some of the demonstrated effects of THF are at least partially due to its metabolites. For example, it is reasonable to conclude that the CNS effects of THF are due at least in part to the activity of GHB. In addition, there is some support for metabolite effects on decreased adult body weight and thymus effects. On the other hand, the limited comparison data provide no evidence that the effects of THF on the liver, kidney, or postnatal development are due to the action of metabolites. The critical effect from the THF oral data is delayed postnatal development (delayed eye and auditory canal opening in male pups) with liver was a cocritical effect for THF in mice following inhalation exposure. Therefore, while metabolites of THF may be more potent at inducing some effects of THF, this might not

hold true for the target tissue toxicity. Therefore, the oral data for THF, and not a metabolite, are most appropriate to serve as the basis for deriving the chronic RfD.

Table 5-1. Comparison of target organ toxicity for THF and metabolites

Target organ	THF	GBL	GHB
CNS	No effect in rat drinking water study at 882 mg/kg-day. Narcosis observed in inhalation studies at estimated systemic doses of 2260 mg/kg-day in mice[a] and 5822 mg/kg-day in rats[b].	Lethargy in rat and mice subchronic gavage at 225 mg/kg-day; EEG changes beginning at 50 mg/kg i.p. in rats in mode-of-action studies	Dizziness in human clinical studies at 12.5 mg/kg LOAEL
Liver	No effect in rat drinking water study at 788 mg/kg-day. Increased absolute and relative liver weight in mice in the inhalation study at estimated systemic dose of 753 mg/kg-day.	No effect in gavage study at 900 mg/kg-day (rats) and 1050 mg/kg-day (mice)	No data
Kidney	Increased kidney weight in rat drinking water study at 714 mg/kg-day.	No effect in gavage study at 900 mg/kg-day (rats) and 1050 mg/kg-day (mice)	No data
Thymus	No oral data. Decreased thymus weight at 753 mg/kg-day and thymus atrophy at 2260 mg/kg-day in mouse inhalation study.	Thymus depletion at 262 mg/kg-day in mouse gavage study[c]	No data
Body weight	Minimally decreased body weight in rat drinking water study at 714 mg/kg-day.	Decreased body weight in rat gavage study at 450 mg/kg-day and in mice at 262 mg/kg-day	No data
Development	Decreased pup body weight gain and delayed eye and auditory canal opening at 782 mg/kg-day in rat drinking water study. Fetal weight, skeletal alterations in rat inhalation studies.	No effects in rat gavage at 500 mg/kg-day	No data
Reproductive	No effect in rat drinking water study on reproductive function or testes weight at 788 mg/kg-day.	Decreased testes weight in rat gavage study at LOAEL of 667 mg/kg-day	No data

[a]For this cursory analysis, estimated systemic doses were calculated from the inhalation studies assuming 100% absorption and EPA default parameter values for mice as follows: LOEL exposure concentration (mg/m^3) × default EPA ventilation rate (0.063 m^3/day) × study exposure duration (6 hours/24 hours) /default EPA body weight (0.037 kg) = mg/kg-day.

[b]For this cursory analysis, estimated systemic doses were calculated from the inhalation studies assuming 100% absorption and EPA default parameter values for rats as follows: LOEL exposure concentration (mg/m^3) × default EPA ventilation rate (0.36 m^3/day) × study exposure duration (6 hours/24 hours) /default EPA body weight (0.38 kg) = mg/kg-day.

[c]No effects on thymus weight were observed in the 13-week study (NTP, 1998). Thymus histopathology in the chronic study (NTP, 1998) was attributed by the authors to injuries secondary to fighting.

One uncertainty in comparing the target organ toxicities of THF and its metabolites is that the metabolites appear to be more potent than THF in inducing CNS effects. Oral dosing data for humans are not available for THF for a direct comparison with the CNS effects of GHB in humans. Since human effects are most relevant for deriving an RfD, an argument could be

made that it would be appropriate to derive the chronic RfD for THF from the CNS effects levels observed in humans exposed to GHB. This could be accomplished by further developing the human PBTK model for THF (Droz et al., 1999) to estimate the oral dose of THF in humans that corresponds to the LOAEL of 12.5 mg/kg for GHB. However, as noted above, uncertainty would remain regarding the potential for THF to induce effects on the liver, kidney, and postnatal development, and, therefore, the oral dosing study for THF is still preferred for deriving the chronic RfD.

Another consideration that supports the use of the THF oral study for derivation of the chronic RfD is the potentially important role of the dosing regimen on the induction of CNS effects. The human clinical studies make it clear that for the CNS effects of GHB, bolus dosing regimens have an important effect. For example, as shown in Table 5-2, large differences in total daily dose did not show a significant change in overall response rate and severity when the individual doses were similar (Gallimberti et al., 1993, 1992). Furthermore, the incidence of effects and their severity generally corresponds to the individual doses rather than the total daily dose (Nimmerrichter et al., 2002; Gallimberti et al., 1993).

Table 5-2. Comparative effects of single and multiple daily dosing

Reference	Single dose (mg/kg)	Maximum daily dose (mg/kg-day)	Effect
Gallimberti et al. (1993)	25	300	Dizziness (5/41)
Gallimberti et al. (1992)	17	50	Dizziness (3/41)
Addolorato et al. (1998)	50	150	Vertigo and lethargy (30% of 109 patients)
Nimmerrichter et al. (2002)	10–20	50	Vertigo (9/31); majority after the double dose
	20–40	100	Vertigo (17/33); seizure (1/33); disorientation (1/33)—majority after the double dose
Scharf et al. (1998)	30	60	Altered brain wave measurements during sleep

Peak doses rather than cumulative doses appear to drive the CNS response, and, therefore, the human studies and animal gavage studies are unlikely to represent the dose response for THF following oral dosing that more closely resembles expected human patterns of THF exposure. The absence of observed CNS effects in the two-generation drinking water study in rats (Hellwig et al., 2002; BASF, 1996) at higher daily doses than those for GBL, which also caused CNS effects (NTP, 1992), is reasonably explained by differences in exposure pattern. Continuous drinking water exposures might not result in sufficient peak levels of exposure to induce the CNS effect. Other explanations exist for the absence of reported CNS effects in the two-generation study. For example, this study did not look in detail at neurobehavioral effects (only clinical signs were evaluated) and may not have provided a sensitive analysis of

neurotoxicity potential. Nevertheless, since the oral exposure route for the BASF study (Hellwig et al., 2002; BASF, 1996) most closely parallels likely human exposure and a clear impact of peak exposure is suggested by the available human data for GHB, this study is the most appropriate basis for deriving the chronic RfD for THF.

In summary, although THF is apparently well absorbed by either the oral or inhalation routes of exposure, tissue distribution studies for THF by both routes of exposure are not directly comparable. It is likely that first-pass metabolism effects may occur and cannot be addressed by the data available. Therefore, deriving a chronic RfD based on extrapolation from the inhalation data is not supported. Analysis of the toxicity data for THF metabolites do not account for the potential effects of the parent molecule. Accordingly, the data from the BASF (1996) and Hellwig et al. (2002) drinking water study is the most appropriate for deriving the RfD of THF. Nevertheless, this study (Hellwig et al., 2002; BASF, 1996) is minimally sufficient to derive the chronic RfD for two reasons. The oral two-generation study did not evaluate all the potential toxic endpoints of interest as identified from the inhalation studies. While the oral two-generation study did evaluate two sensitive endpoints, clinical signs of CNS toxicity and liver histopathology, and found no effects of THF, these results does not rule out the possibility that the other effects observed following inhalation exposure (e.g. thymus weight changes), which were not tested in the BASF study (BASF,1996; Hellwig et al., 2002), could be observed following oral exposure. Second, the subchronic NTP (1998) study suggests that mice might be more sensitive than rats to some of the effects of THF, although clinical signs of CNS toxicity might be a strain, rather than species-dependent response. Therefore, the BASF study (BASF, 1996; Hellwig et al., 2002), which used rats, might not have been the most sensitive indicator of some of the demonstrated systemic effects of THF.

5.1.2. Methods of Analysis

Potential critical endpoints from the two-generation reproduction study (Hellwig et al., 2002; BASF, 1996) were evaluated by using the benchmark dose (BMD) modeling approach, and the final selection of the point of departure for deriving the chronic RfD was made after the evaluation of effect levels among multiple endpoints as described below.

5.1.2.1. *Benchmark Dose Approach*

The kidney weight data for F0 generation animals, the adult (F0 female), and F1/F2 pup body weight gain, as well as other developmental toxicity data in the two-generation reproduction study (Hellwig et al., 2002; BASF 1996), were further analyzed to determine BMD estimates for these endpoints. The observed increases in F0 adult kidney weight do not display a

dose response, so BMD estimates were not derived from these data sets. The observed decreases in body weight in F0 and F1 adults were of questionable biological significance; therefore, these data were not modeled. Visual inspection of the data set for delayed eye opening in F2 pups suggested that the results were not amenable to modeling, and therefore this endpoint was also not modeled. The F1 and F2 pup weight gain data were deemed suitable for BMD estimates for these endpoints. The modeling was conducted according to draft EPA guidance (U.S. EPA, 2000c) by using Benchmark Dose Software (BMDS) version 1.3.2, available from EPA (U.S. EPA, 2001). All references to "polynomial" in Table 5-3 represent revision 2.3, intermediate to the recently released BMDS version 1.4.1. A summary of the results is shown in Table 5-3 (the methods used, models selected, and full copies of the model output have been placed in Appendix B). Only models suitable to the observed data were considered for empirical fits. At the first cut, the criterion to determine whether the models are suitable is conformance of shape.

Table 5-3. Benchmark dose modeling results for two-generation reproductive toxicity study, developmental effects

Endpoint and model	Fit df	AIC[a]	*p*-Value/ degree of freedom	BMD_{1SD}[b]	$BMDL_{1SD}$[b]	$BMD_{0.5}$	$BMDL_{0.5}$
Pup body weight gain F1 males (compare to 0, 128, 364, 1020)[a]							
Power (power 1)	3	309.31	0.53/2	888	614	715	440
Polynomial	4	311.40	0.24/1	909	611	749	437
Pup body weight gain F1 females (compare to 0, 128, 364, 1020)							
Power (power 1)	3	298.74	0.28/2	898	621	691	458
Polynomial	4	300.87	0.20/1	897	617	687	455
Pup body weight gain F2 males (compare to 0, 125, 373, 941)							
Power (power 1)	3	197.80	0.50/2	861	601	552	309
Power (any)	4	199.80	0.24/1	861	595	552	260
Polynomial	4	199.92	0.22/1	873	597	583	307
Pup body weight gain F2 females							
Power(power 1)[c]	3	204.54	0.29/2	940	642	434	305
Power (any)	4	206.97	0.16/1	881	413	298	45
Polynomial[c]	3	204.97	0.23/2	940	642	434	305
Pup body weight gain F2 males and females							
Power (power 1)	3	401.78	0.20/6	885	671	439	326
Power (any)	4	403.78	0.13/5	885	628	439	214
Polynomial	3	401.80	0.27/6	877	670	417	326

[a]AIC = Akaike Information Criterion (see Appendix B). The "compare to" values are the doses (mg/kg-day) at which the study was run.
[b]BMDL = 95% lower bound of the BMD. Subscript denotes the specified benchmark response level. All units are mg/kg-day.
[c]This pair provides the same curve.

Sources: Hellwig et al. (2002), BASF (1996).

All of the data sets for pup body weight gain during days 7–14 showed adequate visual and statistical fit by at least one of the models that was run (the Hill model was not run owing to the number of estimated parameters; see Table 5-3). The dose-response pattern was generally similar across the data sets. Ideally, a nested analysis approach would be used in conducting dose-response analyses for developmental endpoints to control for litter-based effects. While the EPA BMDS can run nested analyses for quantal data, the current software does not provide nested analyses for continuous variables. Although a nested model was not used, visual inspection of the data does not suggest that the observed decrease in pup body weight gain is being influenced by only a few litters. Under this condition, conducting the BMD analysis directly from the concentration mean values, without a nested analysis, appears appropriate.

In developing a BMD approach, several other modeling alternatives were considered. For example, a judgment was needed as to whether it would be suitable to combine the data for males and females rather than modeling them separately. To make this determination, a dose-specific pair-wise comparison was made for responses between males and females for pup body weight gain. For F1 pups, the responses at each dose were significantly different between males and females. Therefore, combining F1 male and female data sets would not be appropriate. However, for F2 pups, the responses were comparable between males and females at all doses. BMD analysis of the combined data was performed, and the results are summarized in Table 5-3. It is not clear if the observed sex dependence for F1 but not F2 has a biological basis or reflects only statistical considerations. Since a biological basis for the sex difference cannot be excluded, it is most appropriate to consider the data for males and females separately. Furthermore, the BMD and BMDL estimates for the combined F2 data were similar to the BMD and BMDL estimates derived for either sex individually (see Table 5-3).

The impact of using a percent decrease in pup body weight gain as the benchmark response (BMR) for the critical effect was considered. EPA's BMD technical guidance (U.S. EPA, 2001) recommends selecting a BMR criterion based on the biological considerations for defining an adverse effect for continuous endpoints and, in the event there is no generally accepted biological criterion, presenting the analysis using 1 standard deviation. Decreased pup body weight gain as low as 5% relative to controls was in the experimental range of the data. For pup body weight gain decreases induced by THF, these two alternative BMR criteria provided very similar BMD and BMDL estimates across models, the former around the high dose and the latter generally between that and the mid dose. Since the selections are similar reading down the columns (see Table 5-3), the generation-sex-model combination corresponding to the least Akaike Information Criterion (AIC) (see Appendix B) among the adequate model fits was used. Data corresponding to the F2 males, described by a restricted power model, provided the best fit; the BMD_{05} was 552 mg/kg-day and its $BMDL_{05}$ was 309 mg/kg-day. For purposes of comparison, 1 and 10% BMR results are presented in Appendix B.

5.1.2.2. *Selection of the Critical Effect Level*

Both increased kidney weight and decreased pup body weight gain were observed at the same concentrations in a two-generation reproductive and developmental toxicity study (Hellwig et al., 2002; BASF, 1996).

Increased relative kidney weight was observed at similar doses in the F0 males and females in both the one- and two-generation studies. Treatment-related effects on absolute kidney weight were less severe. For example, the only group for which both relative and absolute kidney weights were significantly increased ($p < 0.05$) was F0 males in the two-generation study, although apparent increases (that were not statistically significant) were noted in other groups. The observation that, at least for one group, both absolute and relative liver weights were increased can add weight to an argument that these changes reflect the effects of THF on the kidney itself and are not due solely to body weight changes. This conclusion is supported by the general absence of an effect of THF on body weight gain in adult animals.

Although the data indicate that THF induced an increase in kidney weight in rats, the severity of the impact on the kidneys appears to be minimal. This conclusion is supported by several considerations as discussed in detail in Section 4.3.1. THF exposure had no effect on absolute or relative kidney weight in F1 generation adults. Furthermore, the kidney weight changes that were observed in the F0 generation were not accompanied by gross kidney pathology or hematology or clinical chemistry findings consistent with an effect on renal function (in the one-generation study) or by histopathological examination (in the two-generation study). Evaluation of the overall database for THF, including inhalation studies, does not suggest that THF is a potent kidney toxicant. For example, most of the available human case reports have not identified the kidney as a target of THF exposure. Furthermore, in the subchronic and chronic inhalation NTP (1998) studies, changes in kidney weight or pathology were not particularly sensitive to THF exposure. Furthermore, the mode-of-action data are limited and provide useful information regarding the potential severity of the kidney weight changes.

Changes in pup body weight gain observed in the two-generation study represent an effect that is appropriate as the basis for the quantitative assessment (see Section 4.3.1). Changes in pup body weight gain were consistently observed in both the F1 and F2 generation pups and in F2 pups were accompanied by other developmental delays (i.e., delayed opening of the eyes and auditory canal in male pups). These changes occurred in the absence of significant maternal body weight changes or other overt signs of systemic toxicity. Thus, the $BMDL_{05}$ of 309 mg/kg-day for decreases in F2 male pup weight gain was used as the basis for the chronic RfD.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The $BMDL_{05}$ of 309 mg/kg-day for reduced pup weight gain in male Wistar rats exposed throughout gestation and lactation was selected as the point of departure in the derivation of the chronic RfD (Hellwig et al., 2002; BASF, 1996).

A total UF of 1000 was applied to the point of departure of 309 mg/kg-day: 10 for intraspecies variability (UF_H: human variability), 10 for interspecies differences (UF_A: animal to human), and 10 to account for database deficiencies (UF_{DB}).

A 10-fold UF_H was applied to account for human variability in susceptibility among members of the human population (interindividual). Although a human PBTK model (Droz et al., 1999) is available, human variability related to toxicokinetic data for THF in humans is not completely understood.

A UF_A of 10 was applied to account for uncertainty in extrapolating from animals to humans. There are some data to suggest minimal differences between rats and humans with regard to the toxicokinetics. However, the data are not adequate to provide a quantitative estimate of these toxicokinetic differences. In addition, no data are available to evaluate toxicodynamic differences between rats and humans.

No UF was applied to account for extrapolation from a subchronic-to-chronic exposure. Decreased pup body weight gain was chosen as the critical effect. A UF was not used to account for extrapolation from less-than-chronic results because developmental toxicity was used as the critical effect. The developmental period is recognized as a susceptible life stage where exposure during certain time windows is more relevant to the induction of developmental effects than lifetime exposure. However, the two-generation reproduction study (Hellwig et al., 2002; BASF, 1996) reported treatment-related effects in both parental animals and their offspring. In adult animals, increased kidney weights were observed at termination of the subchronic exposure regimen. The observed kidney effects were of questionable biological significance to serve as the basis for deriving a chronic RfD but should be considered in evaluating the appropriate factor for extrapolation from the two-generation study. Interim sacrifice data were not available in the two-generation studies, and therefore, in the absence of a chronic study, it is not possible to determine directly from the current data whether kidney weights would change with increasing exposure duration. The absence of information on the mechanism underlying the kidney weight change also makes it difficult to judge the likelihood that kidney effects would progress with longer durations of exposure. In the absence of data from the oral database, information from the subchronic and chronic inhalation studies (NTP, 1998) can be useful for assessing the progression of systemic effects. The inhalation data (see Section 4.2) did not suggest that the critical effects for the inhalation route (clinical signs of CNS effects and liver toxicity) progress with exposure duration. Since the inhalation and oral dosing effects (at least those likely to be attributed to the parent compound) would be expected to be qualitatively similar and no evidence of effect progression for systemic effects was observed in the inhalation studies, it is reasonable

to conclude that the kidney effects also would not progress. There is uncertainty in this judgment, since no evidence of significant noncancer THF-related kidney toxicity was observed in the inhalation studies, and, therefore, potential progression of kidney effects in the oral studies cannot be judged directly.

A UF_{DB} of 10 was applied to account for database deficiencies. Based on the currently available studies, the oral database is minimally adequate to characterize the oral toxicity of THF. For THF, the only available oral studies were a one-generation dose range-finding study (BASF, 1994) and a follow up two-generation reproduction study (Hellwig et al., 2002; BASF, 1996). While the two-generation study included exposures for approximately 90 days and reported results of histopathologic examinations of the liver, kidney, digestive tract, and reproductive organs, it did not include the comprehensive examination of all organs and systems that would be done in a well-conducted subchronic bioassay. Therefore, even if the two-generation study in rats is considered equivalent to a full subchronic study, the oral toxicity database is lacking a general systemic toxicity study in a second species. The database is also lacking adequate oral developmental toxicity studies in two species. This data gap is considered significant since the critical effect observed in the available oral toxicity studies was decreased pup body weight gain.

The absence of systemic toxicity data for a second species is considered significant, since the spectrum of effects observed in the inhalation studies was different in mice than in rats, with mice being the more sensitive species. For example, a comparison of the NOAEL in rats in the subchronic inhalation study (NTP, 1998) of 5310 mg/m^3 to the NOAEL for mice of 1770 mg/m^3 in this same study suggests that species differences exist. Although the inhalation data support the oral assessment, potential differences in kinetics and uncertainty regarding the identity of the toxic moiety for the critical effect in the oral study preclude using the inhalation data directly to reduce the database uncertainty factor. Similarly, the oral testing data for THF metabolites and related mode-of-action data support the oral data for THF but do not replace it. For example, oral studies for GBL, a metabolite of THF, did not identify kidney or developmental effects, suggesting that these effects might be related to the parent compound and not the metabolites.

Based on limitations in the extent of tissues evaluated in the two-generation study (Hellwig et al., 2002; BASF, 1996), the absence of an adequate general long-term study in another species, the absence of adequate developmental toxicity studies following oral dosing, and uncertainty regarding the potential for immune effects, a factor of 10 is used to account for insufficiencies in the database.

A UF to account for extrapolation from a LOAEL to a NOAEL was not applied, since BMD modeling was used to determine the point of departure.

The chronic RfD based on the $BMDL_{05}$ for decreased pup body weight gain (Hellwig et al., 2002; BASF, 1996) can be derived as follows:

$$\text{RfD} = \text{BMDL}_{05} \div (\text{UF}_H \times \text{UF}_A \times \text{UF}_{DB})$$
$$= 309 \text{ mg/kg-day} \div (10 \times 10 \times 10)$$
$$= 309 \text{ mg/kg-day} \div 1000$$
$$= 0.3 \text{ mg/kg-day}$$

5.2. INHALATION REFERENCE CONCENTRATION

5.2.1. Choice of Principal Study and Critical Effect with Rationale and Justification

Many human occupational exposure studies and case reports investigate the health effects following inhalation exposure to THF. These studies indicate that the nervous system and liver are the primary targets of toxicity of THF. In general, all of the human studies contain insufficient data on the duration and/or concentration of THF exposure. In addition, the human inhalation exposure studies indicate the potential for coexposure to other solvents, most of which have been shown to affect the nervous system. Therefore, reliable effect levels cannot be identified from the available reports of human experimental and occupational exposure. Although several subchronic inhalation studies of THF are available (see Table 4-12), the most reliable inhalation toxicity studies of THF are the subchronic and chronic studies with rats and mice conducted by NTP (1998). Of all the inhalation toxicity studies available, the NTP (1998) subchronic and chronic studies in rats and mice were well conducted and reported, used adequate numbers of animals, evaluated all organs and systems, and conducted the most complete examination of the respiratory tract.

In the subchronic NTP (1998) study, F344 rats and B6C3F1 mice (50/sex/group) were exposed to 0, 66, 200, 600, 1800, and 5000 ppm (0, 195, 590, 1770, 5310, and 14,750 mg/m^3) THF for 6 hours/day, 5 days/week for 13 weeks. No respiratory tract effects were observed in either species following either subchronic or chronic exposure. In both male and female rats exposed to THF for 13 weeks (NTP, 1998), effects, including narcosis, altered organ weights (thymus, spleen, and liver), and altered hematological parameters were observed at the high dose. No nonneoplastic lesions were observed in both male and female rats following the 13-week THF exposure study. The NOAEL for rats is 5310 mg/m^3 and the LOAEL is 14,750 mg/m^3.

In male mice, absolute and relative liver weights were significantly increased and absolute and relative thymus weights were significantly decreased following subchronic exposure to concentrations of greater than or equal to 1770 mg/m^3. Clinical signs of CNS toxicity (narcosis) were observed in male mice in this subchronic study (NTP, 1998) following exposure to 5310 mg/m^3. Effects at the high concentration of 14,750 mg/m^3 included narcosis and liver cytomegaly in both males and females, degeneration of adrenal cortex in female mice only, and uterine atrophy. Based on clinical signs of CNS toxicity in male mice and supported

by liver toxicity, the NOAEL in the subchronic mouse study was 1770 mg/m^3 and the LOAEL was 5310 mg/m^3.

In the chronic NTP (1998) study, F344 rats and B6C3F1 mice (50/sex/group) were exposed to 0, 200, 600, and 1800 ppm (0, 195, 590, 1770, and 5310 mg/m^3) THF for 6 hours/day, 5 days/week for 105 weeks. Significantly decreased survival was observed in male mice at the high dose compared with the controls. The decrease survival was attributed to an increased incidence of urogenital tract inflammatory lesions. No effects were observed in males at 1770 mg/m^3, and no significant nonneoplastic effects were observed in female mice at any concentration in this 2-year study. Clinical signs of CNS toxicity (narcosis) were the only nonneoplastic effect observed in male mice exposed to THF at 1800 ppm (5310 mg/m^3). Based on these effects in males, the LOAEL is 5310 mg/m^3 and the NOAEL is 1770 mg/m^3.

In summary, the NTP (1998) study was selected as the principal study for conducting the quantitative assessment. The NOAEL for clinical signs of CNS toxicity was 1770 mg/m^3 (600 ppm) with a LOAEL of 5310 mg/m^3 (1800 ppm). THF also induced a concentration-dependent increase in liver weight in male mice in this study. The increase in relative liver weight was statistically significant beginning at 195 mg/m^3 (200 ppm). A statistically significant increase in nonneoplastic liver lesions (centrilobular cytomegaly) was observed only in the high-concentration group of 14,750 mg/m^3 (5000 ppm). The CNS effects identified a clear NOAEL and LOAEL boundary. The toxicological significance of the observed liver weight changes was considered to be uncertain at the low concentrations, where they were of minimal severity and were not accompanied by other signs of liver toxicity. The liver effects at higher concentrations (liver weight accompanied by early histopathology findings) were judged to be cocritical with the CNS effects for derivation of the RfC. Based on this analysis, the most appropriate value to serve as the point of departure for the quantitative assessment would be a NOAEL of 1770 mg/m^3 for clinical signs of CNS effects and liver toxicity in male B6C3F1 mice (NTP, 1998).

Clinical signs of irritation as well as histopathological changes in the respiratory tract were observed in several acute studies (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983) and one subchronic study (Kawata and Ito, 1984) at lower exposure concentrations than those that induced CNS effects in the NTP (1998) study. Since respiratory tract changes were observed at lower concentrations than clinical signs of CNS toxicity, studies evaluating this endpoint as a potentially critical effect following inhalation exposure to THF were evaluated more closely for their appropriateness to serve as the basis for the RfD.

There were two primary reasons for selecting the NTP study for the derivation of the RfC over the studies reporting irritation effects: (1) the apparent transient nature of the irritation effects and (2) concerns about study design and documentation for the respiratory effect studies. The NTP (1998) study, which covered a broad exposure concentration range, did not report histopathology changes consistent with respiratory tract toxicity at experimental concentrations up to 5000 ppm. Furthermore, respiratory tract effects consistent with irritation have been

demonstrated to decrease with exposure duration. For example, Horiguchi et al. (1984) reported nasal histopathology after a 3-week exposure to 100 or 5000 ppm THF but no such effect following exposure to 5000 ppm for 12 weeks. The authors reported the tendency for apparent alleviation of irritation effects with repeated exposure. Taken together, these results demonstrate that the irritation effects induced by THF are transient in nature.

The studies that identified respiratory effects at low concentrations were also given less weight than the NTP (1998) study in selecting the critical effect due to concerns about study quality or design. The respiratory effect study that identified the lowest adverse effect level was conducted by Horiguchi et al. (1984), who reported that rats exposed to 100 ppm THF for 3 weeks had changes in the nasal mucous membrane that were similar to those observed in the tracheal mucosa. Changes in the tracheal mucosa in the group exposed to 5000 ppm were described as occurring in the cilia, with disorder of the epithelial architecture and darkening of cell bodies. However, the study authors did not clarify whether the nasal effects at 100 ppm were the same as the tracheal effects at 100 ppm or at 5000 ppm, although it was presumed that it was the tracheal effects at 5000 ppm that were being equated to the 100 ppm nasal effects. The authors did not describe any results for the tracheal mucosa at 100 ppm. A deficiency in this study is that the results represent a single animal per exposure level at each time point. Based on the small sample size, duration of exposure, absence of clear documentation of the severity of the nasal histopathology, and uncertainty regarding the concentration at which nasal changes were observed, this study would not be judged as appropriate to serve as the basis for the RfC.

Liver and thymus weight changes in male mice exposed subchronically to THF were the only other effects observed at the low doses in the NTP (1998) study. Since THF induced nonneoplastic histopathology changes in the liver in mice (NTP, 1998) and may induce liver toxicity in humans (Garnier et al., 1989; Edling, 1982), it is important to determine whether early changes in liver weight are consistent with an adaptive response. NTP (1998) showed that increased relative liver weight (statistically significant) in male mice was significantly increased (by 7%) beginning at 200 ppm (590 mg/m^3) and absolute liver weight was significantly increased (by 7%) at 600 ppm (1770 mg/m^3). Both relative and absolute liver weight increases were also observed in female mice beginning at 1800 ppm (5310 mg/m^3). The only treatment-related, statistically significantly increased incidence of histopathology in the liver was centrilobular cytomegaly in males, which was reported in 7/10 animals at 5000 ppm (14,750 mg/m^3). However, the incidence of centrilobular cytomegaly was 1/10 male mice in the 1800 ppm exposure group (5310 mg/m^3) and this effect was considered to be treatment related based on the general correspondence between centrilobular cytomegaly and increased liver weight across the entire concentration range. No signs of degenerative changes in the liver (e.g., necrosis) were observed, and clinical chemistry results such as serum levels of hepatic enzymes were not measured.

Mode-of-action studies conducted to determine the mode of action for liver tumors in female B6C3F1 mice also provide useful information in weighing the adversity of the nonneoplastic liver changes. A reevaluation of the liver tissues from the subchronic NTP (1998) study using PCNA staining did not reveal a significant increase in cell proliferation in the livers of females mice (BASF, 1998). In contrast to this finding, BASF (2001a) reported increased cell proliferation (particularly in zone 3) in females mice exposed to an average THF concentration of 1794 ppm (5382 mg/m^3) for 5 or 20 days. Coincident with the increase in cell proliferation, exposure to THF at this concentration also generated a statistically significant increase in CYP450 content and activity. In a follow-up study to evaluate the role of CYP450 in the induction of liver toxicity (BASF, 2001b), increased cell proliferation and CYP450 induction were observed in female mice exposed to 14,739 mg/m^3 THF for 5 days. The cell proliferation response was modestly increased in mice pretreated with a CYP450 inhibitor. This result suggests that THF is active as the parent compound, at least with regard to the cell proliferation response.

Evaluating the liver effects of THF in the NTP (1998) study in light of the mode-of-action data (BASF, 2001a,b, 1998) assists in determining whether increased liver weight is more consistent with an adaptive or toxic effect. Two plausible explanations exist for the increase in liver weight—induction of CYP450 and increased cell proliferation. Increased liver weight due to CYP450 induction would be consistent with a potentially adaptive effect, while increased liver weight due to cell proliferation would be considered as a potentially adverse endpoint. Increased liver weight and centrilobular cytomegaly (NTP, 1998) are both responses consistent with the ability of THF to induce CYP450 (BASF, 2001a,b; Elovaara et al., 1984). Since no other signs of liver toxicity were observed, the only potential sign of liver toxicity would be increased cell proliferation. Although cell proliferation was not increased in tissues from the subchronic NTP (1998) study, it was increased in short-term mode-of-action studies. This response was enhanced when CYP450 activity was inhibited, suggesting that THF is itself the active moiety. These results suggest that the induction of CYP450 and increases in organ weight are consistent with an adaptive effect (i.e., by increasing the capacity of the animal to handle additional exposure). On the other hand, in female mice, increased cell proliferation (BASF, 2001a) and increased liver weight (NTP, 1998) were observed at the same concentration, possibly indicating that the observed increases in liver weight were secondary to a cell proliferation response. Since the available data do not unequivocally demonstrate the mode of action, the increased liver weight was considered a potentially significant biological effect. The exposure concentration of 1800 ppm (5310 mg/m^3) is the most appropriate to use as a critical effect level, based on statistically significant increases in absolute and relative liver weights of greater than 10% above controls accompanied by minimal increases in histopathology findings (1/10 incidence in centrilobular cytomegaly) and supported by increased cell proliferation at this concentration in acute mode-of-

action studies. Since this exposure level has not been associated with clear functional or degenerative changes in the liver, this effect level should be considered as minimal in severity.

Thymus weight changes were also a sensitive effect observed in the NTP (1998) subchronic study. Absolute and relative thymus weights were significantly decreased, beginning at 600 ppm (1770 mg/m^3) in male mice. The thymus weight changes were not accompanied by histopathological changes in the subchronic study. The study authors indicated that the significance of the thymus weight changes were unclear and suggested that these changes might have been due to stress associated with THF administration. However, the thymus weight changes were concentration dependent, suggesting that if they were stress related this response would have been secondary to the effects of THF. Organ weights were not reported for the chronic study, and therefore it is not possible to determine if thymus weight is similarly affected by long-term exposure. Histopathological analysis of the thymus in the chronic study revealed an increase in the incidence of thymic atrophy that was statistically significant in the 1800 ppm (5310 mg/m^3) exposure group. However, this result was attributed by the authors to be a secondary response, based on the high incidence of urogenital inflammation observed in the high-concentration males. However, since the increase in infections occurred in the same group that had thymic changes, it cannot be determined whether the thymus weight and histopathology effects increased susceptibility to infection or the inflammation had a stress-related effect on the thymus.

It is unclear whether the observed effects on the thymus in the subchronic and chronic studies (NTP, 1998) represent a functional effect on the immune system, and no data are available to differentiate between a mechanism involving a generalized stress response versus other mechanisms directly targeting the immune system. Evaluation of the THF database as a whole provides inconsistent results related to immune effects, with some studies identifying effects and others showing no effect. Nevertheless, at least some of the available studies show evidence for potential immunotoxicity. For example, decreased white blood cell counts were reported in a study of workers (Horiuchi et al., 1967) and changes in white blood cell counts were reported in an oral drinking water study (Pozdnyakova, 1965) and in a subchronic inhalation study (Horiguchi et al., 1984). Both thymus and spleen weights were reduced in female rats in the subchronic NTP (1998) study. In addition, data for THF metabolites are consistent with potential immunotoxicity. For example, thymic depletion was reported at 262 mg/kg-day GBL in mice in a gavage study (NTP, 1992), although this may have been secondary to an inflammatory response or a factor leading to the susceptibility to inflammation. The toxicokinetic information also provides a plausible connection between THF exposure and immune effects, in which the tissue distribution study by Kawata and Ito (1984) reported that the thymus and spleen had significantly higher THF concentrations than other tissues following inhalation exposure to 3000 ppm THF for 12 weeks.

The predictivity of thymus weight changes for functional immune responses has been studied by Luster et al. (1992) who determined the ability of a variety of common measures of immune toxicity, including thymus/body weight ratios, to predict the immunotoxicity of a series of test compounds in mice. When evaluated as a single measure, thymus/body weight ratios were characterized as an unreliable indicator of immunotoxicity (68% concordance—the ability to correctly identify compounds of known immunotoxic potential). However, thymus/body weight ratio was part of several testing configurations that showed 100% concordance with immunotoxicity, suggesting that this measure can contribute to the immunotoxicity assessment. In addition, the authors noted that the lack of concordance for most assays was generally due to a decreased sensitivity (i.e., failure to detect positive immunotoxicants) not a decrease in specificity (i.e., the ability to correctly identify negative compounds). This suggests that thymus/body weight ratios might underreport immunotoxicants. In a follow-up paper by Luster et al. (1993), a good correlation was reported between immune function assays and changes in host resistance (e.g., increased susceptibility to infection from a challenge agent), although the predictivity of individual assays varied (the concordance was 76% for thymus/body weight ratios).

In summary, there are no studies of host resistance or data from other types of immunotoxicity studies following inhalation exposure to THF. Also, it is unclear whether the observed thymus weight changes had a functional impact on the immunity of mice in the subchronic study (NTP, 1998). For this reason, the decrease in thymus weight was not considered to be a sufficient response to assign an adverse effect level. A significant area of uncertainty exists for the potential effects of THF on the immune system, specifically with regard to decreased thymus weight.

5.2.2. Methods of Analysis

The BMD modeling approach was used in the derivation of the RfC. Human equivalent concentrations for potential critical endpoints were used in applying the approach and the final selection of the point of departure was made after the evaluation of effect level among multiple endpoints from the principal study (NTP, 1998) as described below.

5.2.2.1. *Calculation of Human Equivalent Concentrations*

In order to determine the gas category for calculation of human equivalent concentrations (HECs), the physical and chemical properties of THF were evaluated. THF is water soluble. The toxicokinetic data indicate that THF is systemically absorbed and widely distributed, following inhalation by the exposure route, in humans (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988; Wagner, 1974) and animals (Elovaara et al., 1984; Kawata and Ito, 1984). This combination of properties is consistent with the classification of THF as a category 2 gas (U.S. EPA, 1994b).

The toxicity data also support the conclusion that THF is a category 2 gas, because it induces both portal-of-entry effects as well as systemic toxicity. With regard to portal-of-entry effects, THF induced a variety of effects on the respiratory tract in inhalation studies. Respiratory tract irritation was reported in human case reports of solvent-exposed workers (Garnier et al., 1989; Emmett, 1976) and in acute inhalation studies in animals (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983; DuPont Haskell Laboratory, 1979; Stasenkova and Kochetkova, 1963; Henderson and Smith, 1936). Histopathological lesions of the respiratory tract were noted in several short-term (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983) and longer-term studies (Kawata and Ito, 1984; Stasenkova and Kochetkova, 1963) but were not observed in several other studies that included longer-term exposure periods (NTP, 1998; Horiguchi et al., 1984; BASF, 1938).

Respiratory tract effects identified in these studies are consistent with portal-of-entry toxicity, because they appeared to be largely irritant in nature and were noted most commonly at high doses or following short periods of exposure. The data are not sufficient to rule out the possibility that respiratory tract effects resulted from THF delivery to these tissues after systemic distribution. However, the absence of reports of clinical signs of respiratory tract toxicity (detailed evaluation of nasal and lung tissues were not reported) in oral dosing studies (BASF, 1996; Komsta et al., 1988) suggests that systemically distributed THF or its metabolites do not affect the respiratory tract. Therefore, based on the available data, it is most appropriate to conclude that THF has some properties of a category 1 gas (highly water soluble, rapidly reactive) as described under current RfC methodology (U.S. EPA, 1994b).

A variety of systemic targets of THF have been identified in inhalation bioassays. For example, the nervous system, liver, thymus, cardiovascular system, CNS, kidneys, and spleen have been shown to be potential targets of THF-induced toxicity (NTP, 1998; DuPont Haskell Laboratory, 1996b; Horiguchi et al., 1984; Kawata and Ito, 1984; Stasenkova and Kochetkova, 1963; BASF, 1938). The induction of these extrarespiratory tract effects is consistent with properties of a category 3 gas as described under current RfC methodology (U.S. EPA, 1994b).

THF induces a spectrum of effects consistent with both category 1 and category 3 gases, and the physical and chemical properties of THF are most consistent with category 2; however, current RfC methodology for category 2 gases is not available (U.S. EPA, 1994 b). Therefore, for the purposes of calculating HECs, respiratory tract effect levels were calculated by using the default equations for category 1 gases and extrarespiratory tract effect levels were calculated using default equations for category 3 gases.

For the category 1 equations, HECs are calculated by multiplying the duration adjusted exposure concentration by the regional gas dose ratio (RGDR) for affected portions of the respiratory tract (i.e., pulmonary, tracheal, bronchial, or extrathoracic). For respiratory tract effects, the RGDR is calculated as follows:

$$RGDR = (MV_a/S_a)/(MV_h/S_h)$$

where

MV_a = animal respiratory minute volume

MV_h = human respiratory minute volume

S_a = surface area of the affected respiratory region for the tested species and strain

S_h = surface area of the affected respiratory region for humans

and therefore, the HEC is calculated as

$$\text{duration-adjusted NOAEL/LOAEL} \times RGDR = NOAEL/LOAEL_{HEC}$$

Only one adequate study of subchronic duration identified respiratory tract effects as a potential critical effect in male Wistar rats (Kawata and Ito, 1984). The HEC based on the equation for a category 1 gas was calculated according to the method described above, and the results are shown in Table 4-12.

For the category 3 equations, HECs for gases are calculated by multiplying the duration-adjusted exposure concentration by the RGDR for the extrarespiratory region. The RGDR for extrarespiratory effects is calculated by finding the ratio of the animal-to-human blood:gas (air) partition coefficients. In cases where there are either no data available or where the animal partition coefficient is larger than the human coefficient, a default value of 1 is used for the RGDR. For THF, a human blood:gas partition coefficient was available from Ong et al. (1991); however, no value was available for animals. Therefore, the default of 1 was applied in estimating the HECs for extrarespiratory effects. For example, for the NOAEL study concentration of 1770 mg/m^3 from the subchronic NTP (1998) the HEC based on the equation for a category 3 gas was calculated as follows:

$$1770 \text{ mg/m}^3 \times 6/24 \text{ hours} \times 5/7 \text{ days} = 316 \text{ mg/m}^3 \text{ duration-adjusted concentration}$$

$$316 \text{ mg/m}^3 \times \text{default RGDR of } 1 = 316 \text{ mg/m}^3 \text{ as the HEC}$$

The HECs calculated for each study concentration were used directly in conducting the benchmark concentration (BMC) modeling, and therefore the resulting 95% lower bound of the BMC (BMCL) estimates are shown in terms of HECs.

5.2.2.2. *Benchmark Concentration Approach*

Based on the analysis of the available inhalation toxicity studies, the best value to serve as the point of departure for the quantitative assessment was a NOAEL of 1770 mg/m^3 for clinical signs of CNS effects and liver toxicity in male B6C3F1 mice (NTP, 1998). The HEC for the NOAEL of 1770 mg/m^3 was calculated as follows: 1770 mg/m^3 × 6/24 hours × 5/7 days = 316 mg/m^3 duration-adjusted concentration and 316 mg/m^3 × default RGDR of 1 = 316 mg/m^3 as the HEC. No incidence data for the clinical signs of CNS toxicity were available from the NTP (1998) study, and therefore BMC modeling could not be conducted for this endpoint. Suitable data appeared available to model the liver weight and liver histopathology findings. BMC modeling was conducted for absolute liver weight, relative liver weight, and centrilobular cytomegaly data sets for male mice in the NTP (1998) study. The modeling was conducted according to draft EPA guidance (U.S. EPA, 2000c) by using BMDS version 1.3.2, available from EPA (U.S. EPA, 2001). U.S. EPA's BMD technical guidance (U.S. EPA, 2001a) recommends selecting a BMR criterion based on the biological considerations for defining an adverse effect for continuous endpoints, and, in the event there is no generally accepted biological criterion, presenting the analysis using 1 standard deviation.

For the absolute liver weight data set, none of the models provided an adequate statistical fit when modeling was done using the full data set. A truncated data set with the highest concentration group data removed, but retaining the LOAEL, could be fitted. However, only one model, an unrestricted power model, fit and its BMC_{1SD} of 9 mg/m^3 and corresponding $BMCL_{1SD}$ of 2 mg/m^3 as HEC were low relative to the study's (NTP, 1998) experimental range.

For the relative liver weight data set, none of the models provided an adequate statistical fit when modeling was done using either the full data set or a truncated data set with the high-concentration group data removed (results are shown for the models examined for the latter). For the relative liver weight data, even the constant-variance Hill model, where as many parameters are fitted as there are dose groups, was sensitive to variance differences across groups. The point of departure could have been identified by modeling the data with the two highest concentration groups removed. However, this approach is equivalent to modeling without the LOAEL.

For the centrilobular cytomegaly data set, the full suite of quantal models in BMDS was run. In all instances, a BMR of 10% extra risk was specified. Although all of the models provided an adequate fit overall to the data set based on a goodness-of-fit *p* value greater than 0.1, not all of these models provided an adequate fit in the range of the BMR, as shown by the visual fit and the chi-square residuals for the low-concentration data points. Among all the available models, two groups were able to fit the data points well in the range of the BMR and therefore were considered for selection of a BMCL (see Table 5-4). The first group (the gamma, log-logistic, log-probit, and Weibull models) had goodness-of-fit values of 1 since at least three parameters had to be fitted to only three points and their BMCs were identical. The goodness-of-

fit values for the second group (the multistage and the quantal quadratic) were greater than 0.9 and their BMCs were identical since the BMCL estimates from these models were all within a factor of three, and AIC from these two groups of models were 22.72 or 20.86, respectively (the AIC compares model fit while controlling for the number of parameters used in each model). These models cannot be differentiated on the basis of the adequacy of the fit. In the first group, the gamma and Weibull models provided the most sensitive BMCL of 266 mg/m^3, with a corresponding BMC of 948 mg/m^3. The second group provided the most sensitive BMCL of 256 mg/m^3, with a BMC of 805 mg/m^3 using the multistage model.

Table 5-4. Noncancer benchmark concentration modeling results for THF

Male mice: absolute liver weight (without highest concentration)				
Endpoint and model	**AIC**	***p*-Value**	**BMC_{1SD}[a]**	**$BMCL_{1SD}$[a]**
Power (unrestricted)[b]	–287.93	0.67	9	2
Power (restricted)	–265.71	<0.00001	259	209
Hill	–281.39	0.007	42	23
Polynomial (degree 2, restricted coefficient)[c]	–267.71	<0.0001	259	209
Polynomial (degree 2, unrestricted)[c]	–275.87	0.002	102	75
Male mice: relative liver weight (without highest concentration)				
Endpoint and model	**AIC**	***p*-Value**	**BMC_{1SD}[a]**	**$BMCL_{1SD}$[a]**
Power (unrestricted)	18.30	<0.00001	1.0	0.2
Power (restricted)	66.62	<0.00001	275	221
Hill	12.25	<0.0001	12	4
Polynomial (degree 2, restricted coefficient)[c]	68.62	<0.0001	275	221
Polynomial (degree 2, unrestricted)[c]	42.50	<0.0001	65	51
Male mice: centrilobular cytomegaly (compare to 0, 948, 2634)[d]				
Endpoint and model	**AIC**	***p*-Value**	**BMC_{10}[a]**	**$BMCL_{10}$[a]**
Gamma (power 1)	22.72	1.0	948	266
Log-logistic (slope 1)	22.72	1.0	948	322
Logistic	23.04	0.66	1138	645
Multistage (coefficients 0)[e]	20.86	0.93	**805**	**256**
Probit	22.89	0.75	1061	602
Log-probit	22.72	1.0	948	358
Quantal linear	23.08	0.36	327	189
Quantal quadratic[e]	20.86	0.93	805	607
Weibull (power 1)	22.72	1.0	**948**	**266**

[a]Concentrations used in the modeling were the HECs reported in mg/m^3.
[b] Results with alternative BMRs for power (unrestricted): BMC_{10}: 790; $BMCL_{10}$: 532; BMC_{05}: 118; $BMCL_{05}$: 53; BMC_{01}: 1.4; $BMCL_{01}$: <1.
[c]All references to "polynomial" in Table 5-4 represent BMDS revision 2.3, intermediate to the version recently released with BMDS version 1.4.1.
[d]The "compare to" values are the doses at which the study was run.
[e]This pair provides the same curve.

Source: Based on data from NTP (1998).

5.2.2.3. *Selection of the Critical Effect Level*

The choice of the point of departure depended largely on the interpretation of the observed liver weight changes. As discussed in Section 5.2.1, the liver weight changes occurring at concentrations lower than the 1800 ppm (948 mg/m^3) should not be considered of sufficient severity to be considered biologically significant, since they were limited in degree and were not accompanied by histopathology or clinical chemistry changes indicative of liver injury. It follows that selecting a BMCL for liver weight in the range of 2 mg/m^3 would not correspond to a toxicologically significant effect level and therefore should not be selected for derivation of the RfC. Thus, the BMC modeling results for increased incidence of centrilobular cytomegaly provided the basis for deriving the RfC. Both the gamma- and Weibull-based $BMCL_{10}$ of 266 mg/m^3 and the multistage-based $BMCL_{10}$ of 256 mg/m^3 are within the experimental range and on the order of 300 mg/m^3, as is the NOAEL, 316 mg/m^3. Since these $BMCL_{10}$ estimates provide a point of departure that is within the range of the LOAEL and the NOAEL for CNS and liver effects observed in NTP (1998), a $BMCL_{10}$ of 266 or 256 mg/m^3 is the most appropriate basis for deriving the RfC. Since these models cannot be differentiated on the basis of the adequacy of the fit, the two values (266 and 256 mg/m^3) were averaged to give 261 mg/m^3 for use in the quantitative dose-response assessment (U.S. EPA, 2001). No incidence data were reported for clinical signs of CNS toxicity in the NTP (1998) study. Therefore, BMD modeling could not be conducted for this endpoint. However, the derivation of the RfD based on the NOAEL of 316 mg/m^3 for CNS toxicity is presented for comparison purposes in Section 5.2.3.

5.2.3. RfC Derivation, Including Application of Uncertainty Factors

The $BMCL_{10}$ of 261 mg/m^3 for increased incidence of centrilobular cytomegaly in male B6C3F1 mice exposed to 0, 195, 590, 1770, 5310, or 14,750 mg/m^3 THF for 6 hours/day, 5 days/week for 90 days was selected as the point of departure in the derivation of the RfC (NTP, 1998).

A total UF of 100 was applied to the point of departure of 261 mg/kg-day: 10 for intraspecies variability (UF_H: human variability), 3 for interspecies differences (UF_A: animal to human), and 3 to account for database deficiencies (UF_{DB}).

A UF_H of 10 was used to account for human variability. Sensitive human populations have not been identified for THF (see Section 4.7). A human PBTK model has been derived for THF (Droz et al., 1999); however, this model fails to adequately describe human variability related to toxicodynamics and toxicokinetics of THF.

A UF_A of 3 was used to account for extrapolation from animals to humans. The interspecies extrapolation factor embodies two areas of uncertainty: pharmacokinetics and pharmacodynamics. In this assessment, the pharmacokinetic component was addressed by the calculation of the HEC according to the procedures in the RfC methodology (U.S. EPA, 1994b).

Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{1/2}$ or approximately 3).

No UF was applied to account for extrapolation from a subchronic-to-chronic exposure. The critical study for THF (NTP, 1998) reported clinical signs of CNS toxicity during the exposure period in both subchronic and chronic studies. Both studies identified the same NOAEL and LOAEL for this effect. The subchronic study also identified increased liver weight as a potential biologically significant effect. However, liver weight was not measured after 2 years of exposure. Since there is a chronic inhalation study for THF that clearly indicates that NOAELs will not be higher following chronic exposure than following subchronic exposure, no UF is needed.

A UF_{DB} of 3 was applied to account for deficiencies in the database for THF. Chronic inhalation bioassays are available in rats and mice (NTP, 1998), and inhalation developmental toxicity studies are available in rats (Mast et al., 1992 ; DuPont Haskell Laboratory, 1980) and mice (Mast et al., 1992). No two-generation study by the inhalation route is available; however, there is a two-generation study of THF in drinking water (BASF, 1996). The inhalation data for THF (see Section 4.2) suggest that fetuses and weanling animals are not likely to be more sensitive than adult animals. This conclusion is supported by the observation that, in the inhalation toxicity database (see Table 4-12), the LOAELs for systemic toxicity in adult animals are significantly lower than the LOAELs for developmental toxicity. However, the inhalation developmental studies are limited, since they did not provide an evaluation of postnatal development. In the only available multigeneration study for THF, postnatal development (decreased pup body weight gain and delayed eye and auditory canal opening) was affected at drinking water concentrations that had minimal effects on the dams. Based on the results from the two-generation study, an argument could be made that the early postnatal period is a period of increased susceptibility, but this conclusion is very uncertain, since the changes in pup body weight are at least partially explained by effects on maternal water intake and food consumption. Furthermore, the related measure of fetal weight was not affected in the inhalation studies or in the oral developmental study for GBL. The critical effect level in mice for CNS toxicity and liver effects was substantially lower than for other endpoints, suggesting that this value may also be adequate to protect against developmental effects. Based on these considerations there is some remaining uncertainty regarding the relative potency of maternal and postnatal development effects following inhalation exposure to THF.

A remaining area of uncertainty exists with regard to immunotoxicity as described in detail in Section 5.2.1 regarding the selection of the critical effect. Significantly decreased thymus weight was observed in the subchronic NTP (1998) study at a threefold lower concentration than the concentration that induced the critical effect (clinical signs of CNS toxicity). The thymus weight changes observed in the subchronic NTP (1998) study were not chosen as the critical effect, since in the absence of other assays no functional impairment was

demonstrated. However, the thymus effects do suggest an area of uncertainty when the following additional findings are considered: (1) increased thymic atrophy in the chronic inhalation THF study (NTP, 1998); (2) increased thymic degeneration in the chronic oral study for GBL (NTP, 1992); (3) sporadic evidence of immune effects reported in several other studies; and (4) preferential THF tissue distribution in the spleen and thymus (Kawata and Ito, 1984). These data provide additional support for the application of a database uncertainty factor. Based on these considerations a UF of 3 is most appropriate.

A UF to account for extrapolation from a LOAEL to a NOAEL was not applied, since BMD modeling was used to determine the point of departure.

The RfC based on the $BMCL_{10}$ for increased incidence of centrilobular cytomegaly in male B6C3F1 mice (NTP, 1998) can be derived as follows:

$$\begin{aligned} \text{RfC} &= BMCL_{10} \div (UF_H \times UF_A \times UF_{DB}) \\ &= 261 \text{ mg/m}^3 \div 100 \\ &= 2.6 \text{ mg/m}^3 \\ &\approx 3 \text{ mg/m}^3 \end{aligned}$$

For comparison, the RfC can be derived from the NOAEL as follows:

$$\begin{aligned} \text{RfC} &= NOAEL \div (UF_H \times UF_A \times UF_{DB}) \\ &= 316 \text{ mg/m}^3 \div 100 \\ &= 3.16 \text{ mg/m}^3 \\ &\approx 3 \text{ mg/m}^3 \end{aligned}$$

5.3. CANCER ASSESSMENT

5.3.1. Choice of Study/Data—with Rationale and Justification

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* following exposure to THF in humans. A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (not significant) and an increased incidence of hepatocellular adenomas and carcinomas in female B6C3F1 mice (significant at the high dose) following inhalation exposure to 200, 600, and 1800 ppm (NTP, 1998) (see Section 4.6.1). The tumor incidences in THF-exposed animals were shown in Table 5-5. No human studies are available on the carcinogenic potential of inhalation exposure to THF. There are no human or laboratory animal data to determine the carcinogenicity of THF by the oral or dermal route.

Table 5-5. Incidences of neoplastic lesions of the livers of female B6C3F1 mice and kidneys of male F344 rats exposed to THF 6 hours/day, 5 days/week for 105 weeks

Lesion	Concentration (ppm)			
	0	200	600	1800
Female B6C3F1 mice				
Hepatocellular adenomas				
Overall rate[a]	12/50	17/50	18/50	31/48
Adjusted rate[b]	35.9%	47.1%	52.5%	76.8%
Terminal rate[c]	8/29 (28%)	14/33 (42%)	11/26 (42%)	23/32 (72%)
Logistic regression[d]	$p < 0.001$	$p = 0249$	$p = 0.188$	$p < 0.001$
Cochran-Armitage test[e,f]	$p < 0.001$	–	–	–
Fisher exact test[e,f]	–	$p = 0.189$	$p = 0.138$	$p < 0.001$
Life table test[g]	$p < 0.001$	$p = 0.313$	$p = 0.119$	$p = 0.001$
Hepatocellular carcinomas				
Overall rate	6/50	10/50	10/50	16/48
Adjusted rate	16.5%	26.3%	30.0%	40.8%
Terminal rate	2/29 (7%)	6/33 (18%)	5/26 (19%)	10/32 (31%)
Logistic regression	$p = 0.012$	$p = 0.234$	$p = 0.229$	$p = 0.014$
Cochran-Armitage test	$p = 0.009$	–	–	–
Fisher exact test	–	$p = 0.207$	$p = 0.207$	$p = 0.011$
Life table test	$p = 0.027$	$p = 0.297$	$p = 0.210$	$p = 0.035$
Hepatocellular adenoma or carcinoma				
Overall rate	17/50	24/50	26/50	41/48
Adjusted rate	46.3%	61.3%	69.1%	93.0%
Terminal rate	10/29 (34%)	18/33 (55%)	15/26 (58%)	29/32 (91%)
Logistic regression	$p < 0.001$	$p = 0.188$	$p = 0.086$	$p < 0.001$
Cochran-Armitage test	$p < 0.001$	–	–	–
Fisher exact test	–	$p = 0.111$	$p = 0.053$	$p < 0.001$
Life table test	$p < 0.001$	$p = 0.253$	$p = 0.056$	$p < 0.001$
Male F344 rats				
Renal adenoma				
Overall rate	1/50	1/50	4/50	3/50
Adjusted rate	8.3%	16.7%	18.8%	18.6%
Terminal rate	1/12 (8%)	1/6 (17%)	0/5 (0%)	0/6 (0%)
Logistic regression	$p = 0.213$	$p = 0.602$	$p = 0.159$	$p = 0.262$
Cochran-Armitage test	$p = 0.224$	–	–	–
Fisher exact test	–	$p = 0.753$N	$p = 0.181$	$p = 0.309$
Life table test	$p = 0.195$	$p = 0.602$	$p = 0.120$	$p = 0.213$
Renal carcinoma[h]				
Overall rate	0/50	0/50	0/49	2/50
Renal adenoma or carcinoma				
Overall rate	1/50	1/50	4/50	5/50
Adjusted rate	8.3%	16.7%	18.8%	38.3%
Terminal rate	1/12 (8%)	1/6 (17%)	0/5 (0%)	1/6 (17%)
Logistic regression	$p = 0.037$	$p = 0.602$	$p = 0.159$	$p = 0.065$
Cochran-Armitage test		–	–	–
Fisher exact test	–	$p = 0.753$N	$p = 0.181$	$p = 0.102$
Life table test	$p = 0.031$	$p = 0.602$	$p = 0.120$	$p = 0.042$

[a]Number of animals with tumors per number of animals examined.
[b]Kaplan-Meier estimated tumor incidence at the end of the study, incorporating an adjustment for intercurrent mortality.
[c]Observed tumor incidence at sacrifice.

[d]Logistic regression test assumes that tumors are not fatal.
[e]Control *p* values are associated with the trend test. *p* Values for exposed groups correspond to pair-wise comparisons between controls and exposed groups.
[f]Cochran-Armitage and Fisher exact tests directly compare the overall incidence rates (N represents negative trend).
[g]Life table test takes into account tumors in animals dying prior to terminal sacrifice as being directly or indirectly the cause of death.
[h]No statistical analyses were presented for male rat renal carcinomas.

Source: NTP (1998).

The cancer bioassay for THF is a well-conducted study showing evidence of increased incidence of tumors in differing sexes of two species. Considering these data and uncertainty associated with the suggestive nature of the tumorigenic response, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Since the data for the kidney effects are considered not to be as robust, only the data set of the combined incidence of hepatocellular adenoma or carcinoma observed in female B6C3F1 mice in the NTP (1998) study were modeled.

As discussed in the cancer weight-of-evidence section (Section 4.6.1), some mode-of-action data suggest that the observed male rat kidney tumors may be due to α_{2u}-globulin accumulation. However, since there are residual uncertainties in this conclusion, as compared with the data requirements for demonstrating the α_{2u}-globulin accumulation mode of action (U.S. EPA, 1991b), these tumors were considered relevant in the quantitative cancer assessment. Increased cell proliferation as a mode of action has also been suggested for the livers of THF exposed mice. However, key precursor events linked to observed cell proliferation have not been clearly identified and the mode of action for liver tumor induction by THF is still unknown. The increased incidence of hepatocellular adenomas and carcinomas in female mice exposed to THF was also considered relevant for assessing human carcinogenic potential. Thus, BMD modeling was performed for both male rat kidney tumors and female mouse liver tumors.

5.3.2. Dose Conversion

THF is water soluble and toxicokinetics information suggests that it is systemically absorbed and widely distributed following inhalation exposure in both humans and animals (Droz et al., 1999; Ong et al., 1991; Kageyama, 1988; Elovaara et al., 1984; Kawata and Ito, 1984; Wagner, 1974).

The liver and kidney tumors observed following inhalation exposure to THF are considered extrarespiratory effects of a category 3 gas as defined by EPA guidance for deriving RfCs (U.S. EPA, 1994b). Experimental exposure concentrations would be adjusted to mg/m^3 (0, 590, 1770, and 5310 mg/m^3), adjusted to a continuous exposure basis (mg/m^3 × 6 hours/24 hours × 5 days/7 days = mg/m^3 × 0.1786 : 0, 105.4, 316.1, and 948.4 mg/m^3), and converted to HECs. For the category 3 equations, HECs for gases are calculated by multiplying the duration-adjusted exposure concentration by the RGDR for the extrarespiratory region. The RGDR for

extrarespiratory effects is calculated by finding the ratio of the animal-to-human blood:gas (air) partition coefficients. In cases where there are either no data available or where the animal partition coefficient is larger than the human coefficient, a default value of 1 is used for the RGDR. For THF, a human blood:gas partition coefficient was available from Ong et al. (1991); however, no value was available for animals. Therefore, the default of 1 was applied in estimating the HECs for extrarespiratory effects.

The category 3 gas equation was selected as the appropriate method of dose conversion because the sites of tumorigenic action of THF are consistent with the expected sites of action of a category 3 gas. All exposure concentrations were converted to HECs prior to BMD modeling in the quantitative cancer assessment.

5.3.3. Dose-Response Data

Benchmark concentration modeling was conducted to identify point-of-departure estimates for the linear quantitative cancer assessment of THF. The results of this analysis are summarized below for the two tumor types that were modeled from the NTP (1998) cancer bioassay. A summary of the results is provided in Table 5-6. These results will be used to identify the starting point for deriving the inhalation unit risk (linear assessment).

Table 5-6. Cancer benchmark concentration modeling results for THF

Endpoint and model	AIC	*p* Value	BMC_{10}[a]	$BMCL_{10}$[a]
Hepatocellular adenoma and carcinoma (female mice)				
Multistage	247.842	0.50	73	52
Renal tubule adenoma and carcinoma (male rats)				
Multistage	84.984	0.59	984	499

[a]Concentrations used in the modeling were the HECs reported in mg/m^3.

Source: Modeling based on data from NTP (1998).

The data set of the combined incidence of hepatocellular adenoma or carcinoma observed in female B6C3F1 mice in the NTP (1998) study and the data on renal tubule adenoma and carcinoma in male F344 rats were successfully modeled using the dichotomous multistage model in BMDS version 1.3.1. In both cases, this model provided a relatively good data fit with the goodness-of-fit *p* values higher than 0.10; consequently, these results were used since there was no compelling biological reason to use another empirical model. For the hepatocellular adenoma or carcinoma data set, the BMC and BMCL are 73 mg/m^3 and 52 mg/m^3, respectively. For the renal tubule adenoma and carcinoma data in male F344 rats, the model gives the BMC of 984 (mg/m^3) and corresponding BMCL of 499 (mg/m^3).

Adequate BMCL results were available for both tumor types that were modeled. Thus, the data for female mouse liver tumors were selected for the derivation of the point of departure

for the quantitative concentration-response assessment since they provided the strongest carcinogenic response to inhalation exposure in animals. Therefore, the $BMCL_{10}$ of 52 mg/m^3 was selected as the point of departure for the cancer assessment.

5.3.4. Extrapolation Method(s)

A linear extrapolation from the point of departure to the origin was employed because mode-of-action information for THF is inadequate to inform the low-dose region of the dose-response curve. For THF, the identities of precursor steps leading to cell proliferation are not adequately known. Based on these considerations, the default option, a linear dose-response modeling approach, was chosen as the method of extrapolation from the point of departure.

5.3.5. Oral Slope Factor and Inhalation Unit Risk

Although no human studies were available, a chronic rodent bioassay provides suggestive evidence of THF-induced carcinogenicity (NTP, 1998). The data from this study are adequate to support a quantitative cancer dose-response assessment. Even though the available evidence is suggestive of human carcinogenic potential, there is very limited information exploring the mode of action for any of the tumors reported in the animal chronic studies. THF was not mutagenic or genotoxic in various in vitro studies. In addition, there are inadequate data to support alternative mode-of-action hypotheses. In the absence of such data, extrapolation from the point of departure to lower doses is conducted by using a linear approach. The U.S. EPA *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005) recomend including benign tumors observed in animal studies in the assessment of animal tumor incidence, if such tumors have the capacity to progress to the malignancies with which they are associated. Hepatocellular adenomas have the capacity to progress to hepatocarcinomas and they were therefore combined in the quantitative assessment of the carcinogenic potential of THF in humans.

The inhalation unit risk is derived from the BMC_{10} (the lower bound on the exposure associated with a 10% extra cancer risk) by dividing the risk (as a fraction) by the BMC_{10} and represents an upper bound, continuous lifetime exposure risk estimate. The HEC $BMCL_{10}$ for extra risk of hepatocellular adenomas or carcinomas in female B6C3F1 mice exposed to THF results in an inhalation unit risk of 0.1/(52 mg/m^3) or 0.002 (mg/m^3)$^{-1}$. This value was derived by linear extrapolation to the origin from the point of departure of 52 mg/m^3 and represents an upper-bound estimate. The slope of the linear extrapolation from the central estimate is 0.1/(73 mg/m^3) or 0.0014 (mg/m^3)$^{-1}$.

5.3.6. Confidence in the Cancer Assessment

The principal study (NTP, 1998) used to derive the cancer risks is a well-conducted and documented study. The study included chronic exposure in two species by the relevant route of

exposure, evaluated a comprehensive array of tissues, and covered an appropriate concentration range. It is accordingly well suited to modeling using the BMC approach.

The study (NTP, 1998) concluded that there was *some evidence* of carcinogenic activity for male rats (renal tubule adenoma and carcinomas), *no evidence* of carcinogenic activity for female rats and male mice, and *clear evidence* of carcinogenic activity in female mice (hepatocellular adenoma and carcinoma).

The mode of action of THF-induced kidney and liver tumors is not completely understood. The lack of activity in a number of genotoxicity assays indicates that a genotoxic mode of carcinogenic action is most likely not responsible for the tumorigenic activity of THF. Some mode-of-action data suggest that the observed renal tumors in the male rats may be secondary to α_{2u}-globulin accumulation, a mode of action not relevant to humans. However, no histopathological lesions characteristic of this mode of action were observed. According to the current EPA technical report (U.S. EPA, 1991b), the presence of pathological lesions is a required element for concluding that α_{2u}-globulin accumulation is the mode of action for kidney tumors, and, therefore, in the absence of these pathological findings, the male rat tumors are presumed relevant for the cancer risk assessment. It is noteworthy, however, that the technical report (U.S. EPA, 1991b) was based largely on patterns of effects observed for more potent inducers of α_{2u}-globulin accumulation than THF appears to be and may not account for subtle effects caused by less potent inducers of this mechanism. A single, unpublished report submitted to EPA by the Tetrahydrofuran Task Force of the Synthetic Organic Chemicals Manufacturers Association presented the hypothesis that perhaps advanced CPN may play a role in the incidence of ATH and the development of kidney tumors in male rats following exposure to THF (see Appendix A). However, in the published NTP (1998) 2-year carcinogenicity study there were no differences in the incidence or severity of CPN in the control and high-dose males. Thus, the exact mode of action of THF for the male rat kidney tumors is still unclear.

For the liver tumors in mice, mode-of-action data suggest that THF may induce cell proliferation and lead to a promotion in the growth of preinitiated cells. There was no clear increase in the incidence of foci, although a marginal increase in eosinophilic foci was reported at the high concentration in the NTP (1998) bioassay. The limited effect on foci formation suggests that THF does not act early in the carcinogenesis process and is therefore not a potent tumor initiator. Rather, the absence of an increase in foci coupled with the observed increase in mostly adenomas is more consistent with the promotion of preinitiated cells by THF. In support of the conclusion that THF may have the greatest impact at the promotion stage, THF-induced tumors only occurred in the liver, a tissue that has a high background incidence in B6C3F1 mice. In addition, time to tumors was generally late in life (time at first incidence 399 days versus 478 days for controls), although some concentration-dependent decrease in the time to tumor was observed (NTP, 1998). Appearance of tumors only in tissues with a high background incidence and occurrence primarily as benign tumors late in life are characteristics consistent with tumor

promotion (U.S. EPA, 2005a). However, key precursor events linked to observed cell proliferation have not been clearly identified. This is a major area of uncertainty in the cancer dose-response assessment. Identification of key precursor events responsible for observed cell proliferation is necessary to select the most appropriate dose-response model. The increased incidence of malignant tumors (carcinomas) suggests a possible role in tumor progression as well.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Oral Noncancer

The database for oral toxicity of THF is very limited. Based on the limited data, CNS toxicity appears to be the primary health effect following acute exposure, although no CNS effects were reported in the longer-term oral studies. Longer-term oral studies (4 weeks to approximately 90 days) suggest that general toxicity (characterized by altered food and water consumption and decreased body weight) and liver and kidney toxicity are potential effects of THF. However, no human data were available to confirm that this same spectrum of effects is likely to be observed in people following exposed by this route.

The two-generation reproductive toxicity study (BASF, 1996) was the only oral-dosing study for THF that included a sufficient period of dosing to meet the requirements for a subchronic study. The chronic toxicity and prenatal developmental toxicity of THF metabolites have been tested (reviewed in NSF, 2003). However, uncertainties regarding the dosing methods that were used and the identity of the active moiety for the critical effect in the two-generation study limit the direct use of these metabolite studies. Based on these considerations, the two-generation oral study (BASF, 1996) was selected as the principal study for the derivation of the chronic RfD, with additional supporting documentation provided by the longer-term inhalation studies and oral dosing studies for THF metabolites. However, the available data were considered minimally adequate to derive the chronic RfD. The inhalation studies identify some potential target organs of THF exposure that were not evaluated in the BASF (1996) study. This uncertainty is increased, since some effects of THF were only observed in mice in the inhalation studies, a species that was not evaluated in the oral study (which used rats). THF also induces portal-of-entry effects that might argue against relying on inhalation data for assessing the spectrum of effects likely to be induced by oral exposure. In addition, the available toxicokinetic data were not sufficient to make qualitative judgments on potential oral toxicity, since the tissue distribution studies are not directly comparable, and likely first-pass metabolism effects following oral dosing could not be addressed.

The primary data need in order to increase confidence in the chronic RfD is a long-term oral study in mice that includes a thorough examination of all major tissues. Studies in multiple species are needed, and in particular an oral dosing study in mice would be warranted based on observations from the inhalation data, that mice may be more sensitive to some effects of THF (NTP, 1998). A comprehensive toxicokinetic study in mice and rats has been done following dosing by the oral route (DuPont Haskell Laboratory, 1998). This study suggests some kinetic differences among rodent species. For example, the T_{max} was lower for mice than for rats, and

the percent of the oral dose excreted as CO_2 was greater for mice than for rats. This latter result is consistent with in vitro microsomal metabolism data (DuPont Haskell Laboratory, 2000) that suggest that there are intrinsic differences in metabolic capacity among species. The implications of these toxicokinetic differences with regard to susceptibility to THF are unclear, since the mode of action for each of the effects of THF is not known and no data on toxicodynamic differences among species are available.

The RfD of 0.3 mg/kg-day is based on a $BMDL_{05}$ of 309 mg/kg-day for decreased pup body weight gain (BASF, 1996). A composite uncertainty factor of 1000 was used. This factor is based on a default factor of 10 to account for intrahuman variability, a default factor of 10 to account for extrapolation from an animal study, and a factor of 10 to account for uncertainties in the database. Although the principal study (BASF, 1996) was considered of good quality, the overall confidence in the oral THF database was low, with several key data gaps identified, including a full systemic toxicity study in a second species and developmental toxicity studies in two different species. These gaps were addressed to some degree by qualitative information from the inhalation studies for THF and oral studies of THF metabolites. Therefore, the confidence in the RfD can be characterized as low.

6.1.2. Inhalation Noncancer

Although no epidemiological studies of THF have been conducted, several occupational exposure case studies in humans suggest that target organs in humans are the CNS, respiratory tract, liver, and kidney (Garnier et al., 1989; Albrecht et al., 1987; Juntunen et al., 1984; Edling, 1982; Emmett, 1976). The major uncertainty associated with all of the reported human case studies is the fact that workers were exposed to other solvents and chemicals in addition to THF, so it is not possible to conclusively attribute the observed effects to THF exposure. Nevertheless, inhalation studies in animals generally identified a similar array of target organs (see Table 4-12) and identify clinical signs of CNS toxicity and liver toxicity as the most appropriate choices for the critical effects.

Respiratory tract irritation was reported in multiple human and animal studies. One consideration in evaluating the potential health consequences due to THF-induced respiratory tract irritation is the role of the exposure duration on the severity of the effect. Several short-term studies (Ikeoka et al., 1988; Horiguchi et al., 1984; Ohashi et al., 1983) identified concentrations inducing irritant responses that were lower than the concentrations that induced toxicity in the better documented subchronic and chronic studies (NTP, 1998). There is direct evidence that respiratory tract responses are transient in nature, waning with increasing exposure duration (Horiguchi et al., 1984). These data suggest that irritant responses not observed with subchronic or chronic exposure could occur in individuals who were not previously exposed.

Human case reports and inhalation studies in animals identified the liver as a potential target for the toxicity of THF. The most sensitive liver response in adequate subchronic or

chronic studies was increased liver weight in male mice (NTP, 1998). These early changes might reflect either adaptive or precursor effects for toxicity, and the available data are not sufficient to determine which categorization is most appropriate. Evaluating the liver effects of THF in the NTP (1998) study in light of the mode-of-action data (BASF, 2001a,b, 1998) suggests two plausible explanations for the increase in liver weight: CYP450 induction and increased cell proliferation. Increased liver weight due to CYP450 induction would be consistent with a potentially adaptive effect, while increased liver weight due to cell proliferation might be considered to be of potential biological significance. The available data are equivocal for the mode of action for increased liver weight. Thus, liver weight was considered further as a potential critical effect. Since the liver weight changes observed at low exposure concentrations have not been associated with clear functional or degenerative changes in the liver, this effect would most appropriately be considered as minimal in severity.

The principal study (NTP, 1998) for the RfC identified thymus weight changes in male mice at a lower concentration than those that induced the critical effect (clinical signs of CNS toxicity). The toxicological significance of the thymus weight changes is a significant area of uncertainty, although several lines of evidence support the need to further clarify the immunotoxic potential of THF. This uncertainty could best be alleviated with functional immunotoxicity testing.

The RfC of 3 mg/m^3 is based on clinical signs of CNS toxicity and liver toxicity in male mice in an NTP (1998) study, with a $BMCL_{10}$ of 261 mg/m^3 for increased incidence of hepatocellular centrilobular cytomegaly. A composite uncertainty factor of 100 was used. This factor is based on a default factor of 10 to account for intrahuman variability, a default factor of 3 to account for extrapolation from an animal study for which effect levels were adjusted by appropriate animal-to-human dosimetry, and a factor of 3 to account for uncertainties in the database.

The data were not adequate to move away from the default value of 10 to account for variability in human susceptibility. No sensitive subpopulations have been identified. The existing data do not provide convincing evidence for age- or gender-related differences in sensitivity to noncancer effects of THF, although there is uncertainty regarding the ability of THF to affect postnatal development. However, a number of findings raise questions about the potential for increased susceptibility based on gender, including potential effects in the uterus of mice, apparent sex-specific tumor formation, and toxicokinetic differences between male and female rodents. The absence of adequate human data and uncertainties regarding the role of THF metabolism in the observed toxicity preclude the development of alternative UF values directly from the data.

The principal study used to derive the RfC (NTP, 1998) was a well-conducted and documented study. The study included subchronic and chronic exposure duration components in two species by the relevant route of exposure, evaluated a comprehensive array of tissues, and

covered an appropriate concentration range. Therefore, the identified NOAEL/LOAEL boundary and BMCL were determined with high confidence. Confidence in the supporting database is medium to high. Although chronic toxicity studies (NTP, 1998) and developmental toxicity studies (Mast et al., 1992; DuPont Haskell Laboratory, 1980) were available for the inhalation route, no multigeneration study by the inhalation route is available. Both the inhalation developmental toxicity studies (Mast et al., 1992; DuPont Haskell Laboratory, 1980) and the oral two-generation study (BASF, 1996) show that effects in fetuses and pups occur at doses that cause at least minimal maternal effects and that these doses are much higher than the NOAEL for organ weight changes in mice from the NTP (1998) study. Because an inhalation two-generation study would not be likely to identify a NOAEL lower than that identified by NTP (1998), this area of the database is adequately addressed. Although the standard array of toxicity testing has been adequately addressed for THF, a remaining area of uncertainty exists with regard to its immunotoxic potential. Based on high confidence in the well-conducted critical study and medium confidence in the database, the confidence in the RfC can be characterized as medium to high.

6.1.3. Cancer

No human studies were identified that evaluated the carcinogenic potential of THF via the oral, inhalation, or dermal routes of exposure. In addition, no oral exposure carcinogenicity studies in animals were identified.

The weight of evidence suggests that THF is carcinogenic for more than one species, sex, and site; therefore, there is *suggestive evidence of carcinogenic potential* following exposure to THF in humans (U.S. EPA, 2005). This descriptor is consistent with the NTP (1998) weight-of-evidence evaluation. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of THF is deemed appropriate. EPA concluded that the cancer bioassay available for THF is a well-conducted study showing tumors in two species and two sexes. In addition, a quantitative analysis may be useful for providing a sense of the magnitude and uncertainty of carcinogenic potential risk. Since the data for the kidney effects are considered not to be as robust, only the data set of the combined incidence of hepatocellular adenoma or carcinoma observed in female B6C3F1 mice in the NTP (1998) study were modeled. . The U.S. EPA *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005) recomend including benign tumors observed in animal studies in the assessment of animal tumor incidence, if such tumors have the capacity to progress to the malignancies with which they are associated. Hepatocellular adenomas have the capacity to progress to hepatocarcinomas and they were therefore combined in the quantitative assessment of the carcinogenic potential of THF in humans.

Specifically, no studies evaluating the carcinogenicity of THF by the oral or inhalation route were identified in humans. A 2-year NTP (1998) inhalation cancer bioassay reported an increased incidence of renal tubule adenomas and carcinomas in male F344/N rats (not

significant) and an increased incidence of hepatocellular adenomas and carcinomas in female B6C3F1 mice (significant at the high dose) following inhalation exposure to 200, 600, and 1800 ppm. Data for female mouse liver tumors were selected as the basis for the derivation of the inhalation unit risk because this was the strongest carcinogenic response to inhalation THF exposures observed in animals.

For the male rat kidney tumors, there is some evidence suggesting the involvement of α_{2u}-globulin, a mode of action not relevant to humans. However, there are remaining uncertainties in conclusively demonstrating this mode of action according to an EPA technical report (U.S. EPA, 1991b). For example, pathology findings secondary to α_{2u}-globulin have not been demonstrated in existing studies, and absence of evaluation of α_{2u}-globulin accumulation and potential precursor events (e.g., cell proliferation) in female rats hinders the determination of the response specificity. For the liver tumors in mice, mode-of-action data appear to suggest that THF may induce cell proliferation and lead to a promotion in the growth of preinitiated cells. However, key precursor events linked to observed cell proliferation have not been clearly identified for THF. This is a major area of uncertainty in the cancer dose-response assessment. Identification of key precursor events responsible for observed cell proliferation is necessary to select the most appropriate dose-response model. In the absence of these data, the default linear dose-response modeling approach was selected.

Exposure concentrations were adjusted to HECs prior to BMD modeling according to EPA (U.S. EPA, 1994b) default dosimetric equations for a category 3 gas. The tumors observed in the kidney and liver following inhalation exposure to THF are consistent with the expected site of action for a category 3 gas. The data set of the combined incidence of hepatocellular adenoma or carcinoma observed in female B6C3F1 mice in the NTP (1998) study were modeled with all the dichotomous models available in the current BMDS version 1.3.2. Concentrations associated with a 10% extra risk for tumors at the lower 95% confidence bounds for the animal curves were determined. The $BMCL_{10}$ of 52 mg/m^3 for hepatocellular adenomas and carcinomas was selected as the point of departure for the quantitative cancer assessment. A linear extrapolation from origin to the point of departure resulted in the derivation of an inhalation unit risk of 0.0019 $(mg/m^3)^{-1}$, which represents an upper bound estimate for human situations.

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APPENDIX A:
A SUMMARY OF THE REANALYSIS OF THE PATHOLOGY OF THE NTP (1998) SLIDES BY THE TETRAHYDROFURAN TASK FORCE OF THE SYNTHETIC ORGANIC CHEMICALS MANUFACTURERS ASSOCIATION

Background

The NTP (1998) study kidney slides were rigorously analyzed by a group of pathologists and this report has been published in the peer-reviewed literature. Against a background of nephropathy that was uniform across all groups, there were more renal tubular tumors in treated rats than in the controls, and those in the higher doses were larger in size. Consideration should be given to the robustness and the gender specificity of the renal tumor response. As such, the NTP (1998) study conclusion (*some evidence* of carcinogenic activity of THF in male rats) is persuasive and provides support for the cancer assessment for THF. Thus, EPA concluded that the male rat kidney tumors were relevant to humans but that the mode of action for these tumors was not completely understood.

Summary

In an unpublished report submitted by the Tetrahydrofuran Task Force of the Synthetic Organic Chemicals Manufacturers Association to EPA, histology slides of kidneys from male and female F344 rats of the 2-year carcinogenicity and 14-week NTP studies (NTP, 1998) with THF were reexamined (Dammann, 2005; Hard, 2005). The authors of these unpublished reports suggested that the overall incidence of kidney tumors in the male rats was 2/50 (4%), 1/50 (2%), 3/50 (6%), and 5/50 (10%), with all tumors being adenomas (Hard, 2005). Use of the Cochran-Armitage trend test on the data presented in the unpublished report showed no significant dose-response trend in tumor incidence (Dammann, 2005).

The author, Hard (2005), also concluded that THF does not appear to act via the α_{2u}-globulin mode of action. Instead, the author proposed that advanced CPN may play a role in the development of ATH, and perhaps the kidney tumors from THF exposure. CPN is an age-related renal disease of laboratory rodents that occurs spontaneously in high incidence. There was no difference in the incidence or severity of CPN in male rats of the NTP 2-year carcinogenicity study on THF (both the control and high-dose groups have 13 males with end-stage kidneys). Although THF did not exacerbate development of CPN, it was postulated that it may have exacerbated the development of proliferative lesions within CPN-affected tissue. No data in the peer-reviewed literature are available that support a role of CPN in the induction of THF-induced kidney tumors in male rats. The exact mode of action of THF for the male rat kidney tumors is still unclear.

APPENDIX B. BENCHMARK DOSE MODELING

The THF data sets considered for dose-response modeling include both quantal and continuous endpoints. EPA's BMDS version 1.3.2 (U.S. EPA, 2001) was used to accomplish most of the model fitting and benchmark estimation. The specific models applied to obtain any comparison values (RfD, RfC, unit risk) are presented here.

Quantal models

All of the quantal models implemented in the BMDS package were used to represent the dose-response behavior of the quantal endpoints. Specifically, the models used were the gamma model, the logistic and log-logistic models, the probit and log-probit models, the multistage model, and the Weibull model (including its special cases, the linear and the quadratic models). The equations defining each of these models are presented here (U.S. EPA, 2000c). In all of the following, P(d) represents the probability of response (i.e., adverse effect) following exposure to "dose" d. In all of these models, α, β, and γ are model parameters estimated using maximum likelihood techniques, as described below.

The gamma model has the form

$$P(d) = \gamma + (1 - \gamma) \times [1/\Gamma(\alpha)] \times \int t^{\alpha-1}e^{-t}dt \qquad \text{Eq. 1}$$

where $0 \leq \gamma < 1$, $\beta \geq 0$, and $\alpha > 0$. $\Gamma(x)$ is the gamma function, and the integral runs from 0 to βd.

The logistic model is defined as follows:

$$P(d) = \{1 + \exp[-(\alpha + \beta d)]\}^{-1} \qquad \text{Eq. 2}$$

where $\beta \geq 0$.

The log-logistic model has much the same form as the logistic model:

$$P(d) = \gamma + (1 - \gamma) \times \{1 + \exp[-(\alpha + \beta \ln(d)]\}^{-1} \qquad \text{Eq. 3}$$

except when d = 0, in which case $P(d) = \gamma$. In this case $\beta \geq 0$, and for the background parameter γ,

$0 \leq \gamma < 1$.

The probit model is given by the equation

$$P(d) = \Phi(\alpha + \beta d) \qquad \text{Eq. 4}$$

and the log-probit model has a similar form:

$$P(d) = \gamma + (1 - \gamma) \times \Phi[\alpha + \beta \ln(d)] \qquad \text{Eq. 5}$$

except when d = 0, in which case $P(d) = \gamma$. Here $\Phi(x)$ is the standard normal cumulative distribution function, $0 \leq \gamma < 1$, and $\beta \geq 0$.

The multistage model is defined as follows:

$$P(d) = \gamma + (1 - \gamma) \times \{1 - \exp[-(\beta_1 d + \beta_2 d^2 + ... + \beta_n d^n)]\} \qquad \text{Eq. 6}$$

where all the β parameters are restricted to be nonnegative and $0 \leq \gamma < 1$.

The Weibull model has the form

$$P(d) = \gamma + (1 - \gamma) \times [1 - \exp(-\beta d^{\alpha})] \qquad \text{Eq. 7}$$

where the background parameter γ is restricted to fall between 0 (inclusive) and 1, and β is greater than or equal to 0. For these analyses, the parameter α is constrained to be greater than or equal to 1.

The linear model is a special case of the Weibull model obtained by fixing the parameter α equal to 1. The quadratic model is a special case of the Weibull model obtained by fixing the parameter α equal to 2. Both the linear and quadratic models, in addition to the unrestricted Weibull model (i.e., in which α was allowed to be estimated), were fit to the quantal THF data sets.

When fitting all of the above-mentioned quantal models, maximum likelihood methods were used to estimate the parameters of the models. That method maximizes the log-transformed likelihood of obtaining the observed data, which is (except for an additive constant) given by

$$L = \sum \{n_i \times \ln[P(d_i)] + [N_i - n_i] \times \ln[1 - P(d_i)]\} \quad \text{Eq. 8}$$

where the sum runs over i from 1 to k (the number of dose groups), and for group i, d_i is the dose (exposure level), N_i is the number of individuals tested and n_i is the number of individuals responding (U.S. EPA, 2000b).

Continuous models

The continuous endpoints of interest were quantitatively summarized by group means and measures of variability (standard errors or standard deviations). The models that can be used to represent the dose-response behavior of those continuous endpoints are those implemented in EPA's BMDS (U.S. EPA, 2001, 2000c). These models include power models (unrestricted or restricted, with a special case, the linear model), Hill models, and polynomial models. These mathematical models fitted to the data are defined here. In all cases, $\mu(d)$ indicates the mean of the response variable following exposure to "dose" d.

The power model can be represented by the equation

$$\mu(d) = \gamma + \beta d^{\alpha} \quad \text{Eq. 9}$$

where the parameter α is nonnegative. When α is unrestricted, it may take on any positive real value. When it is restricted, it must be at least 1. The linear model is obtained when α is fixed at a value of 1.

The Hill model is given by the following equation:

$$\mu(d) = \gamma + (vd^n \div [d^n + k^n]) \quad \text{Eq. 10}$$

where the parameters n and k are restricted to be positive. Because the Hill model has four parameters to be estimated (γ, v, n, and k), as well as the population variance (see discussion of Eq. 12), the model was not fitted to data sets with four dose groups, so that the number of estimated parameters did not exceed the number of data points. Hill models are not well suited to "cup-shaped" data.

The polynomial model is defined as:

$$\mu(d) = \beta b_0 + \beta_1 d + ... + \beta_n d^n \quad \text{Eq. 11}$$

where the degree of the polynomial, n, was set equal to one less than the number of dose groups in the experiment being analyzed. For these analyses, the values of the estimated β parameters were constrained to be either all nonnegative or all nonpositive (as dictated by the data set being modeled [i.e., nonnegative if the mean response increased with increasing dose or nonpositive if the mean response decreased with increasing dose]).

In the case of continuous endpoints, one must assume something about the distribution of individual observations around the dose-specific mean values defined by the above models. Although some endpoints appeared to have dose-dependent variances, where individual observations can be assumed to vary normally around the means with variances given by:

$$\sigma_i^2 = \sigma^2 \times [\mu(d_i)]^\rho \quad \text{Eq. 12}$$

and both σ^2 and ρ are parameters estimated for the model, all fitted models were adequately described by a constant variance across dose groups.

Given these assumptions about variation around the means, BMDS applies maximum likelihood methods to estimate all of the parameters.

Goodness-of-fit analyses

For the quantal models, goodness of fit was determined by BMDS using a chi-square test. That test is based on sums of squared differences between observed and predicted numbers of responders. The degrees of freedom for the chi-square test statistic equals the number of dose groups minus the number of parameters fitted by maximum likelihood (ignoring those parameters that are estimated at a bound defined by their constraints). When the number of parameters estimated equals the number of dose groups, there are no degrees of freedom available for a statistical evaluation of fit.

For the continuous models, goodness of fit was determined based on a likelihood ratio statistic. Here, the ratio's degrees of freedom figure depends on the degrees of freedom of its numerator and denominator. In particular, the maximized log-likelihood associated with the fitted model was compared to a log-likelihood maximized with each dose group having its own mean and variance completely independent of the means and variances of the other dose groups. It is always the case that the latter log-likelihood will be at least as great as the model-associated

log-likelihood, but if the model does a "reasonable" job of fitting the data, the difference between the two log-likelihoods will not be too great. A formal statistical test reflecting this idea uses the fact that twice the difference in the log-likelihoods is distributed as a chi-square random variable. The degrees of freedom associated with that chi-squared test statistic equal the difference between the number of parameters fitted by the model (including σ^2 and ρ when needed to define how variances change as a function of mean response level) and twice the number of dose groups (namely, estimating a mean and variance for each group). This test is denoted "Test 4" in the output produced by BMDS, when dose-dependent variances have been tested, and "Test 3" when a constant variance model is fitted.

Visual fit, particularly in the low-dose region, was assessed for models that had acceptable global goodness of fit. Acceptable global goodness of fit was judged by a *p* value greater than or equal to 0.1 or a perfect fit when there were no degrees of freedom for a statistical test of fit. Local fit was evaluated visually on the graphic output by comparing the observed and estimated results at each data point and by evaluating the chi-squared residuals (continuous data) or scaled residual (quantal data).

Goodness-of-fit statistics are not designed to compare different models, particularly if the different models have different numbers of parameters. (Within a family of models, adding parameters generally improves the fit.) BMDS reports the AIC to aid in comparing the fit of different models. The AIC is defined as $-2L + 2p$, where L is the log-likelihood at the maximum likelihood estimates for the parameters and p is the number of model parameters capable of estimation. When comparing the fit of two or more models to a single data set, the model with the lesser AIC was taken to provide the better fit.

Definition of the BMR and corresponding BMD and BMDL

For all of the quantal endpoints analyzed here, the BMDs and BMDLs were defined based on BMRs of 10% extra risk. BMDLs were defined as the 95% lower bound on the corresponding BMD estimates. Confidence intervals were calculated by BMDS using a likelihood profile method.

Initially for all the continuous models, BMDs were implicitly defined as follows:

$$|\mu(\text{BMD}) - \mu(0)| = \delta \times \sigma_0 \qquad \text{Eq. 14}$$

where σ_0 is the model-estimated standard deviation in the control group. In other words, the BMR was defined as a change in mean $\mu(\cdot)$ corresponding to some multiplicative factor δ of the control group standard deviation. Such a definition can assist for comparisons when an endpoint has no generally accepted BMR.

Values of 1.1 and 1.81 for δ would reflect the work of Crump (1995), who showed that these choices correspond to an additional risk of 10% when the background response rate was assumed to be 1% or 0.1%, respectively, with normal variation around the means (and constant standard deviation). The *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) suggests using $\delta = 1$ as a standard analysis. Additional analyses were carried out for relative deviations of 5 or 10% of the mean.

For all of the BMDs estimated as described above, BMDLs were defined as the 95% lower bound on the corresponding BMD. Confidence intervals were calculated using a profile likelihood method.

Choice of BMDL

EPA guidance was followed with regard to the choice of the BMDL to use as a point of departure. For each endpoint, the following rule of thumb is suggested:

1. Models with an unacceptable fit (including consideration of local fit in the low-dose region) are excluded.
2. If the BMDL values for the remaining models for a given endpoint are within a factor of 3, no model dependence is assumed, and the models are considered indistinguishable in the context of the precision of the methods. The models are then ranked according to the AIC, and the model with the lowest AIC is chosen as the basis for the BMDL.
3. If the BMDL values are not within a factor of 3, some model dependence is assumed, and the lowest BMDL is selected as a reasonable conservative estimate, unless it is an outlier from all of the other models.
4. The BMDL values from all modeled endpoints are compared, along with any NOAELs or LOAELs from data sets that were not amenable to modeling, and the lowest NOAEL or BMDL is chosen.

Endpoints and models used for RfD and RfC

The RfD discussion uses the F2 male rat pup weight gain, power model, with the power restricted to be at least 1. The RfC discussion refers to male mouse liver centrilobular

cytomegaly, with both a gamma model with power at least 1 and a multistage model with nonnegative coefficients. Finally, the inhalation unit risk discussions are based on combined hepatocellular adenomas and carcinomas in female mice, from a quantal linear model.

Pup Body Weight Gain, F2 Male Rats (Hellwig et al., 2002; BASF, 1996)

```
 ================================================================
 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   The form of the response function is:

   Y[dose] = control + slope * dose^power

   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   The power is restricted to be greater than or equal to 1
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                         alpha =       3.1932
                           rho =            0   Specified
                       control =         15.6
                         slope =      20.6019
                         power =    -0.454087

          Asymptotic Correlation Matrix of Parameter Estimates
                  alpha          rho      control        slope        power
    alpha             1           -1        0.051        -0.16        -0.16
      rho            -1            1       -0.051         0.16         0.16

    control        0.051       -0.051            1        -0.59        -0.57
    slope         -0.16         0.16        -0.59            1            1
    power         -0.16         0.16        -0.57            1            1

                         Parameter Estimates
      Variable           Estimate             Std. Err.
         alpha              3.0991              28.4577
           rho                   0              3.24213
       control             17.6071             0.290169
         slope       -4.57291e-005         0.000274311
         power             1.56241             0.867905

     Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	24	17.4	1.56	17.6	1.76	-0.118
125	20	17.9	1.98	17.5	1.76	0.215
373	23	17	1.94	17.1	1.76	-0.0741
941	23	15.6	1.67	15.6	1.76	0.00945

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-95.200286	5	200.400571
A2	-94.325125	8	204.650249
fitted	-95.900074	4	199.800148
R	-105.247729	2	214.495459

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

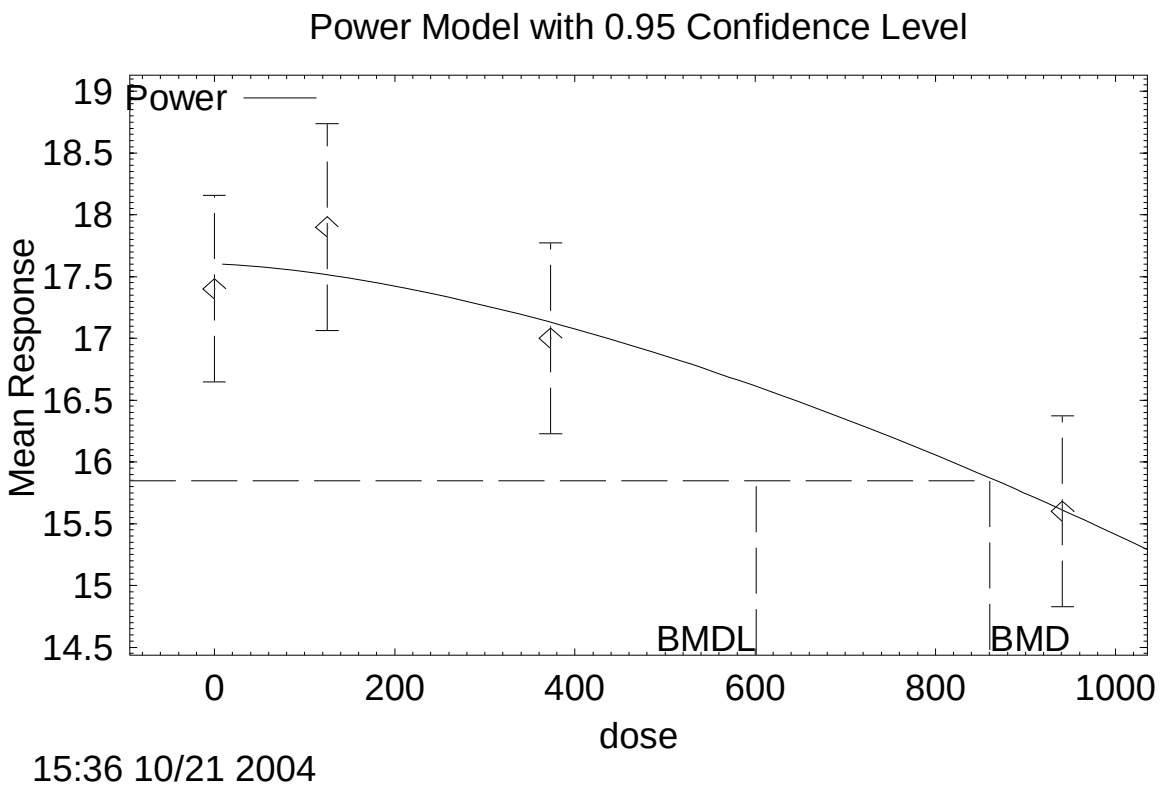
Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	21.8452	6	7.025e-005
Test 2	1.75032	3	0.6258
Test 3	1.39958	1	0.2368

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

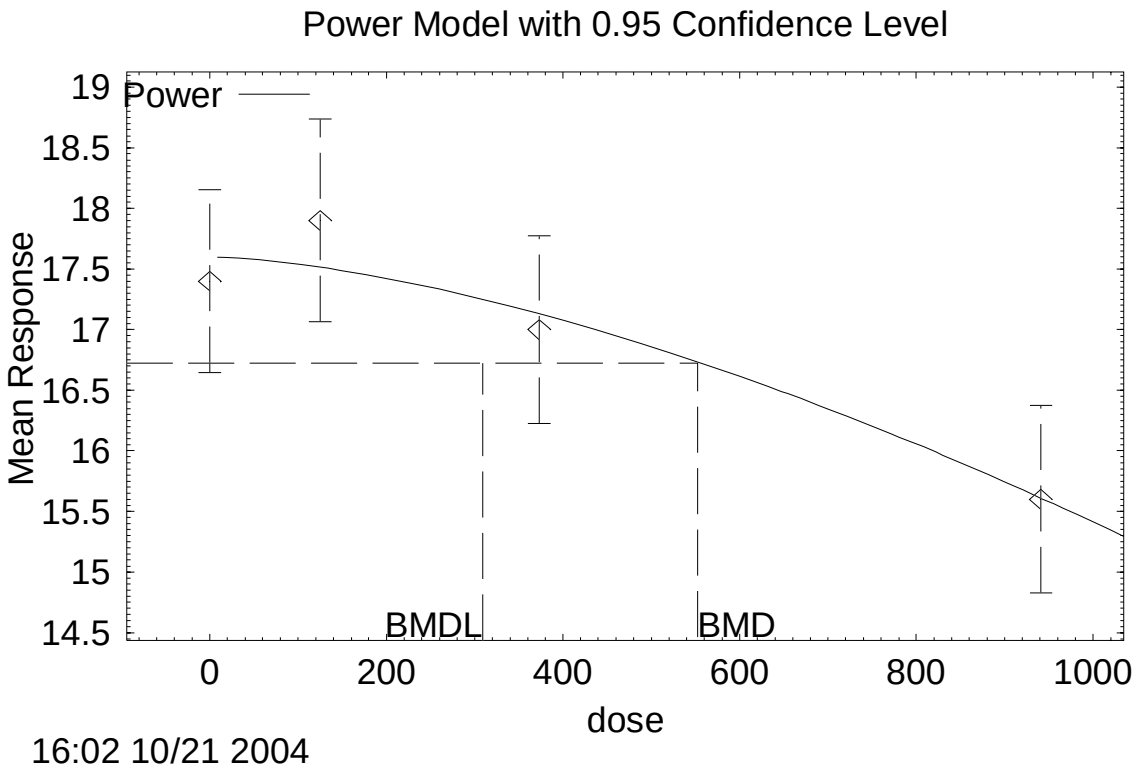
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation
Confidence level = 0.95
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
BMD = 860.692
BMDL = 601.188


Power Model with 0.95 Confidence Level
Power
Mean Response
19
18.5
18
17.5
17
16.5
16
15.5
15
14.5
BMDL
BMD
0
200
400
600
800
1000
dose
15:36 10/21 2004

```
Specified effect =          0.05
Risk Type        =     Relative risk
             BMD =       552.359
            BMDL =       309.325
```



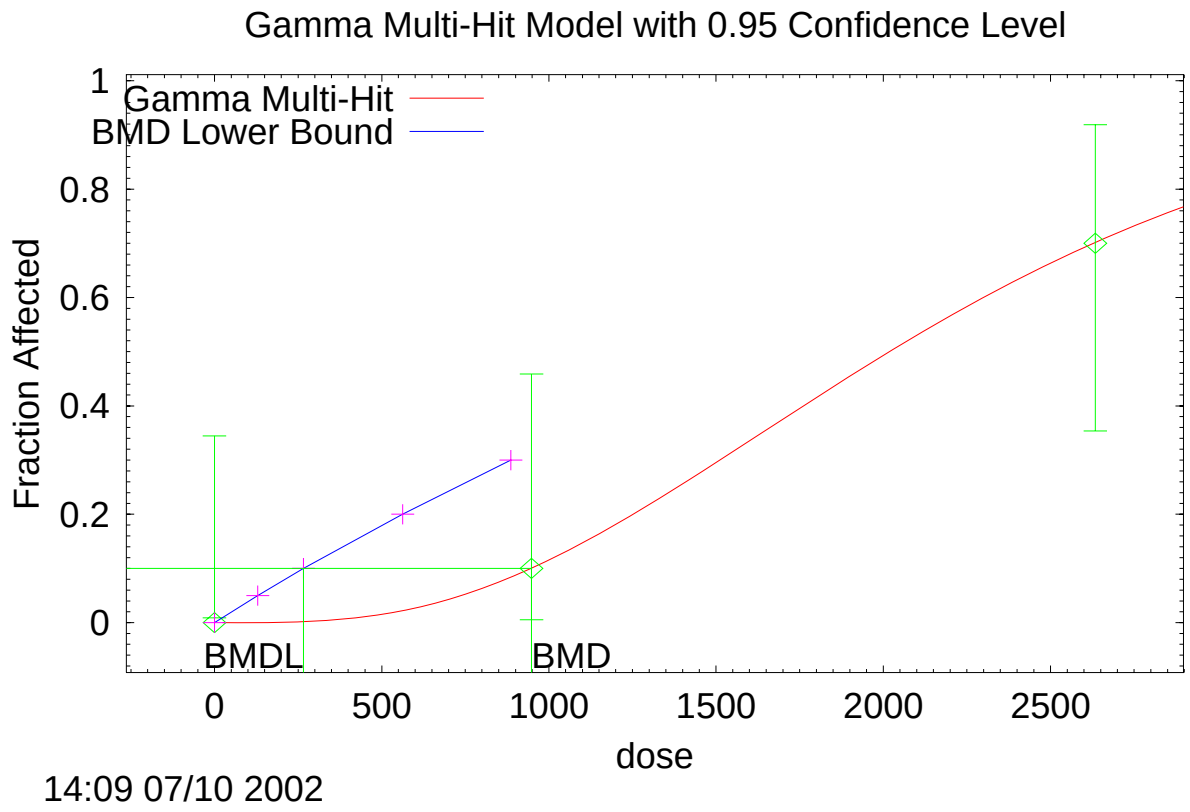

```
Specified effect =          0.01
Risk Type        =     Relative risk
             BMD =       197.176
            BMDL =        61.865
```

```
Specified effect =           0.1
Risk Type        =     Relative risk
             BMD =        860.78
            BMDL =        618.65
```

BMDS MODEL RUN

```
    The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function
   Dependent variable = incidence
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial (and Specified) Parameter Values
                    Background =    0.0454545
                         Slope =   0.00196109
                         Power =       4.4408

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -Background
                have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )
                  Slope        Power
     Slope            1         0.97
     Power         0.97            1

                        Parameter Estimates
       Variable          Estimate            Std. Err.
     Background                 0               NA
          Slope          0.00175608          0.00137574
          Power              3.8798             2.70407

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

                     Analysis of Deviance Table
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -9.35947
   Fitted model        -9.35947  3.41061e-013      1           1
  Reduced model        -17.3975        16.076      2    0.000323
           AIC:         22.7189

                    Goodness  of  Fit
                                                           Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
```

```
    0.0000       0.0000           0.000           0            10             0
  948.0000       0.1000           1.000           1            10   -5.661e-007
 2634.0000       0.7000           7.000           7            10   -1.456e-007

 Chi-square =       0.00     DF = 1        P-value = 1.0000


   Benchmark Dose Computation
Confidence level =           0.95
Specified effect =            0.1
Risk Type        =      Extra risk
             BMD =            948
            BMDL =        265.796
```



BMDS MODEL RUN

The form of the probability function is:

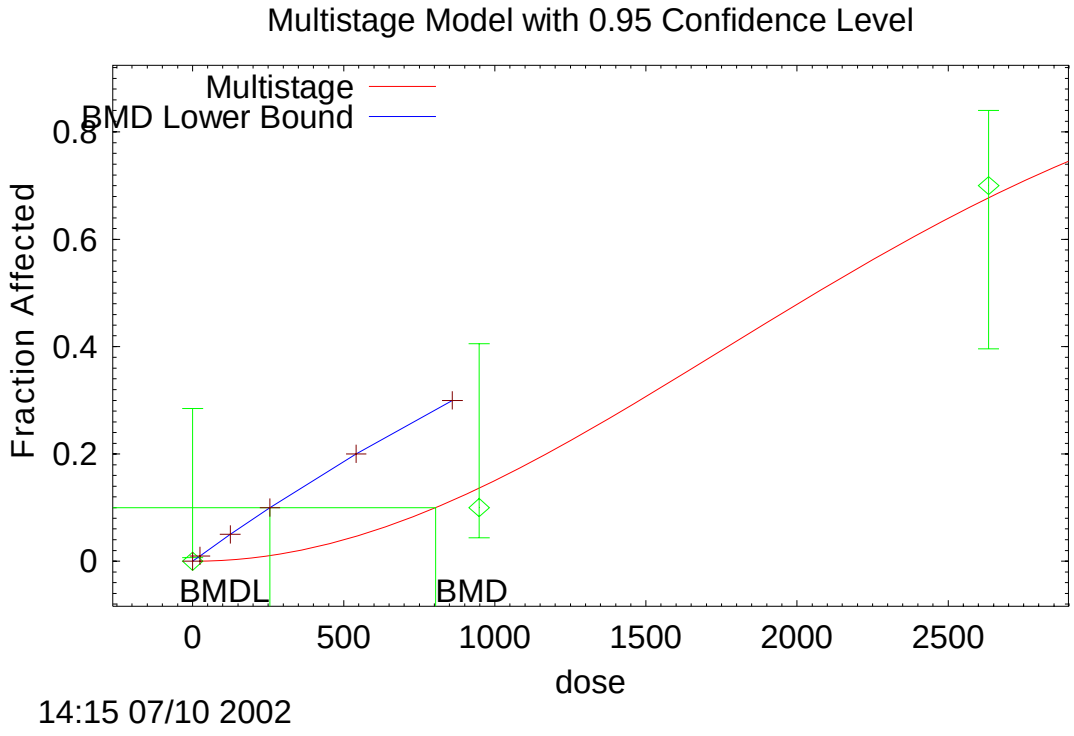
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = incidence

```
   Independent variable = dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) = 1.76579e-007

           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )

               Beta(2)
   Beta(2)           1

                        Parameter Estimates
       Variable          Estimate            Std. Err.
     Background                 0               NA
        Beta(1)                 0               NA
        Beta(2)       1.62776e-007       7.75991e-008

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

                      Analysis of Deviance Table
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -9.35947
   Fitted model        -9.43218     0.145417      2          0.9299
```

```
  Reduced model        -17.3975        16.076      2        0.000323
           AIC:         20.8644

                      Goodness  of  Fit
    Dose      Est._Prob.     Expected     Observed      Size      Chi^2 Res.
-----------------------------------------------------------------------------

   0.0000      0.0000          0.000          0          10        0.000
 948.0000      0.1361          1.361          1          10       -0.307
2634.0000      0.6768          6.768          7          10        0.106
Chi-square =       0.14     DF = 2        P-value = 0.9345

   Benchmark Dose Computation
Confidence level =           0.95
Specified effect =            0.1
Risk Type        =      Extra risk
             BMD =        804.532
            BMDL =           255.
```



Combined Hepatocellular Adenomas and Carcinomas, Female Mice (NTP, 1998)

```
 BMDS MODEL RUN

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]

   Dependent variable = incidence
   Independent variable = dose

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial (and Specified) Parameter Values
                    Background =     0.343137
                         Slope =   0.00153654
                         Power =            1   Specified

          Asymptotic Correlation Matrix of Parameter Estimates
          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user, and do not appear in the correlation matrix )

             Background        Slope
Background            1        -0.52
     Slope        -0.52            1

                         Parameter Estimates
       Variable           Estimate             Std. Err.
     Background            0.345175           0.0534952
          Slope          0.00143523         0.000326562

                       Analysis of Deviance Table
       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -121.226
   Fitted model        -121.921       1.38933      2          0.4992
```

Reduced model	-136.424	30.3951	3	<.0001
AIC:	247.842			

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.3452	17.259	17	50	-0.07697
105.0000	0.4368	21.839	24	50	0.6162
316.0000	0.5839	29.197	26	50	-0.9172
948.0000	0.8320	39.938	41	48	0.4102

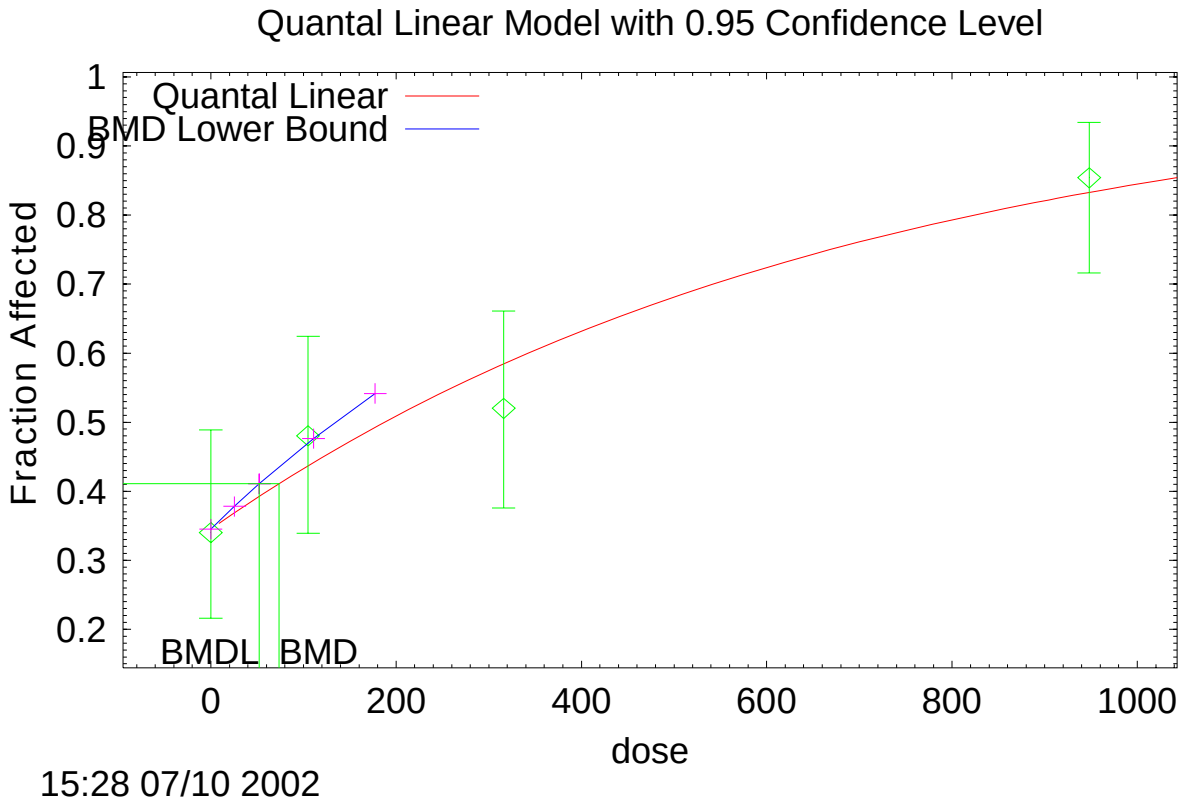
Chi-square = 1.40 DF = 2 P-value = 0.4978

Benchmark Dose Computation

Confidence level = 0.95

Specified effect = 0.1

Risk Type = Extra risk

BMD = 73.4103

BMDL = 52.4022