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Provisional Peer Reviewed Toxicity Values for

4-Chlorobenzotrifluoride
(CASRN 98-56-6)

Superfund Health Risk Technical Support Center
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 4-CHLOROBENZOTRIFLUORIDE (CASRN 98-56-6)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided

in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

No RfD assessment for 4-chlorobenzotrifluoride is available on IRIS (U.S. EPA, 2007) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). The HEAST (U.S. EPA, 1997) lists subchronic and chronic RfD values for 4-chlorobenzotrifluoride of 2E-1 and 2E-2 mg/kg-day, respectively. The source document for these assessments was a Health and Environmental Effects Document (HEED) (U.S. EPA, 1988). Both reference dose (RfD) values were based on a no-observed-adverse-effect level (NOAEL) of 15 mg/kg-day in a study that exposed rats to 4-chlorobenzotrifluoride for 90 days after exposure *in utero* and during lactation (EBL, 1981). The critical effect was tubular degeneration in the kidneys in rats treated with 40 mg/kg-day or more in a different subchronic study (Arthur and Probst, 1983). Uncertainty factors of 100 and 1000 were used to derive the subchronic and chronic RfDs, respectively. Other than the HEED discussed above, the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994a) does not include any relevant documents. The Agency for Toxic Substances and Disease Registry (ATSDR, 2006) and the World Health Organization (WHO, 2006) have not assessed the toxicity of 4-chlorobenzotrifluoride.

An RfC for 4-chlorobenzotrifluoride is not available on IRIS (U.S. EPA, 2007) or in the HEAST (U.S. EPA, 1997). The HEED (U.S. EPA, 1988) reports that there were no pertinent available inhalation toxicity data in humans or animals at the time of publication. Occupational exposure limits for 4-chlorobenzotrifluoride have not been derived by the American Conference for Governmental Industrial Hygienists (ACGIH, 2005), the National Institute for Occupational

Safety and Health (NIOSH, 2006) or the Occupational Safety and Health Administration (OSHA, 2006).

A cancer assessment for 4-chlorobenzotrifluoride is not available on IRIS (U.S. EPA, 2007) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). A cancer weight-of-evidence classification for 4-chlorobenzotrifluoride is not listed in the HEAST. The HEED (U.S. EPA, 1988) assigned 4-chlorobenzotrifluoride to U.S. EPA (1986) Group D (not classifiable as to human carcinogenicity) based on lack of carcinogenicity data via any exposure route. The carcinogenicity of 4-chlorobenzotrifluoride has not been assessed by NTP (2006) or IARC (2006).

Literature searches were conducted from 1988 through October, 2005 for studies relevant to the derivation of provisional toxicity values for 4-chlorobenzotrifluoride. Databases searched included: TOXLINE/TOXCENTER (including BIOSIS and NTIS subfiles), MEDLINE, CANCERLIT, TSCATS, RTECS, CCRIS, DART/ETIC, EMIC/ EMICBACK, HSDB, GENETOX and Current Contents.

REVIEW OF PERTINENT DATA

Human Studies

Relevant data regarding the toxicity of 4-chlorobenzotrifluoride in humans were not located.

Animal Studies

The toxicity of 4-chlorobenzotrifluoride following repeated oral exposure has been studied by NTP (1992), Macri et al. (1987), Arthur and Probst (1983) and EBL (1981), and the subchronic toxicity of 4-chlorobenzotrifluoride following inhalation exposure has been studied by Newton et al. (1998). EBL (1981) also included a reproductive toxicity phase. All of these studies are described below. No chronic toxicity studies via any exposure route were located, and no studies that evaluated the developmental toxicity of 4-chlorobenzotrifluoride via any exposure route were located.

Oral Exposure. 4-Chlorobenzotrifluoride was the subject of a 14-day study conducted by the National Toxicology Program (NTP, 1992; Yuan et al., 1992). Male and female F344/N rats and B6C3F_1 mice (five per sex per dose per vehicle of each species) were given 4-chlorobenzotrifluoride (97% pure) either suspended in α-cyclodextrin (α-CD) or dissolved in corn oil via oral gavage daily for 14 days. The test substance was administered to rats and mice as daily doses of 0, 10, 50 or 400 mg/kg suspended in α-cyclodextrin and as daily doses of 0, 10 (mice only), 50, 400 or 1000 mg/kg dissolved in corn oil. Additional groups of five male rats per dose and vehicle were treated as described above, but used only for determination of α_{2u}-globulin and total protein levels in the kidneys. During the exposure period, all animals were observed twice daily for signs of toxicity, and body weight was recorded every 2 days. All animals were

sacrificed after the 14th treatment. Prior to sacrifice, blood was obtained from all treated and control animals. The following hematology parameters were evaluated (rats only): erythrocyte, leukocyte and platelet counts, hematocrit, hemoglobin concentration, differential leukocyte count and reticulocyte count. The following clinical chemistry parameters were evaluated in both rats and mice: alanine aminotransferase (ALT), total protein, albumin, glucose, triglycerides, cholesterol, urea nitrogen, creatinine, creatine kinase, alkaline phosphatase, 5' nucleotidase, sorbitol dehydrogenase and total bile acids. At sacrifice, a complete necropsy examination was performed; the liver, kidney (right), heart, lungs, thymus and testis (right) were weighed from all treatment and control animals. A comprehensive set of tissues was microscopically examined from all control and high-dose animals and animals that died prior to scheduled sacrifice by two independent pathologists. Only gross lesions, liver, kidney and adrenal glands were microscopically examined in the other rat dose groups, and only gross lesions and the liver were microscopically examined in the other mouse dose groups. 4-Chlorobenzotrifluoride residues were determined in the blood, kidney and liver in rats and mice from all treatment groups (both vehicles). The kidneys were stained to detect the presence of hyaline droplets in the tubular epithelium and lumen. Sections of the liver, kidney and adrenal glands of high dose and control male and female rats were examined by electron microscopy for ultrastructural changes.

Rats showed minimal clinical signs indicative of irritation immediately following dosing (burrowing in bedding, rubbing face with forepaws) with either vehicle (NTP, 1992). One female rat treated with 1000 mg/kg-day in corn oil died after eight treatments. The cause of death was not determined and no other animals died prior to scheduled sacrifice. Final mean body weight was statistically significantly reduced in male rats treated with 1000 mg/kg-day in corn oil; the difference from controls was 6%. Final body weight was similar to controls in the other treated groups. Hematology analyses revealed mild anemia, characterized by slight, statistically significant decreases in erythrocyte counts, hemoglobin and hematocrit, in male and female rats treated with 1000 mg/kg-day in corn oil. The males also showed mild leukocytosis due to increases in segmented neutrophils and monocytes. Minimal changes in some of these parameters were present in the 400 mg/kg-day corn oil groups as well. Serum chemistry changes occurred mainly in the 1000 mg/kg-day group (only corn oil tested) and occasionally in the 400 mg/kg-day groups (both solvents) and included increases in cholesterol, 5'-nucleotidase, bile acids, triglycerides and total protein. Serum cholesterol levels were statistically significantly increased in male rats (α-CD vehicle) at 50 and 400 mg/kg-day (17 and 37% higher than controls), female rats (α-CD vehicle) at 400 mg/kg-day (35% higher), male rats (corn oil vehicle) at 400 and 1000 mg/kg-day (51 and 134% higher) and female rats (corn oil vehicle) at 400 and 1000 mg/kg-day (58 and 119% higher). Serum 5'-nucleotidase activities were increased in male rats (α-CD vehicle) at 50 and 400 mg/kg-day (10 and 15% higher than controls), male rats (corn oil vehicle) at 400 and 1000 mg/kg-day (13 and 62% higher) and female rats (corn oil vehicle) at 400 and 1000 mg/kg-day (21 and 46% higher). Serum levels of bile acids were increased in male rats (corn oil vehicle) at 1000 mg/kg-day (123% higher than controls) and female rats (α-CD vehicle) at 400 mg/kg-day (13% higher). Serum triglyceride levels were increased in female rats (corn oil vehicle) at 1000 mg/kg-day (71% higher than controls). Serum total protein levels were increased in male rats (corn oil vehicle) at 400 and 1000 mg/kg-day (7 and 12% higher than controls) and female rats (corn oil vehicle) at 400 and 1000 mg/kg-day (13 and 17% higher).

Serum alkaline phosphatase and sorbitol dehydrogenase were increased in male rats (corn oil vehicle) at 1000 mg/kg-day (17 and 7% higher than controls).

The only organ weight changes of note were statistically significant increases in absolute and relative liver weights at ≥50 mg/kg-day in males and ≥400 mg/kg-day in females, and absolute and/or relative kidney weights at ≥400 mg/kg-day in males and 1000 mg/kg-day in females (NTP, 1992). Organ weights at a given dose were similar for the different vehicles. Gross pathology examinations revealed enlarged livers in males and females and enlarged kidneys in males at 1000 mg/kg-day. Dose-related microscopic changes in the liver, kidneys and adrenal glands occurred in groups administered the test substance in both vehicles (Table 1). Incidence and severity of these changes increased with increasing dose. In the liver, increased incidences of hepatocyte hypertrophy occurred in male rats at ≥50 mg/kg-day and in female rats at ≥400 mg/kg-day. This effect was described as minimal to mild, consisting of enlargement of hepatocytes in the centrilobular region (involving most hepatocytes within the lobule at the highest dose). Fatty changes were noted in the liver of the 1000 mg/kg-day female rat that died. Nephropathy was observed only in male rats. Severity progressed from minimal to mild at 50 mg/kg-day (primarily accumulation of protein droplets) to moderate at ≥400 mg/kg-day (including degeneration, necrosis and regeneration of tubular cells). Electron microscopic examination revealed focal mineralization along the basal lamina of renal tubules. Kidney levels of α_{2u}-globulin were significantly increased in a dose-related fashion in males treated with ≥50 mg/kg-day in either vehicle. Cytoplasmic vacuolation of the adrenal cortex occurred at ≥400 mg/kg-day in both males and females, although the cause and biological significance of the adrenal lesion were not clear to the investigators.

The hepatocellular hypertrophy occurred at 50 mg/kg-day and higher dose levels, but is not considered to be adverse because it is likely to reflect the proliferation of smooth endoplasmic reticulum and the presumed induction of hepatic microsomal enzymes, which is an adaptive response to chemical exposure. The increases in serum cholesterol, triglycerides, 5'-nucleotidase and bile acids are indicators of cholestasis, generally appeared at 400 mg/kg-day, and were most prevalent at 1000 mg/kg-day. Based on the serum chemistry changes suggestive of cholestatic liver disease, the EPA identified a LOAEL of 400 mg/kg-day and NOAEL of 50 mg/kg-day for rats. The occurrence of adrenal cortex cytoplasmic vacuolation, a possible adverse effect observed at ≥400 mg/kg-day, supports classifying 400 mg/kg-day as the study LOAEL. The renal lesions observed in male rats at ≥50 mg/kg-day are not considered relevant for human health risk assessment. The occurrence only in male rats, nature of the lesions, and correlation with measured levels of α_{2u}-globulin all indicate that the lesions result from accumulation of α_{2u}-globulin in the kidney, which is a toxic response peculiar to male rats and not predictive of renal toxicity in humans (U.S. EPA, 1991b).

In mice, clinical signs of irritation similar to those seen in rats were observed (NTP, 1992). There were no deaths during the study and no effects on body weight. Serum chemistry findings were generally similar to those in rats. The most notable changes were increases in serum cholesterol, 5'-nucleotidase and triglycerides in both males and females. The changes occurred primarily in the 400 mg/kg-day groups (more marked in the α-CD groups than the corn

Table 1. Summary of Microscopic Lesions in Rats Administered 4-Chlorobenzotrifluoride by Oral Gavage for 14 Consecutive Days (NTP, 1992)

Tissue	Lesion	Vehicle	Dose (mg/kg-day)				
			0	10	50	400	1000
Males							
Liver	Hepatocyte hypertrophy	α-CD	0/5[a,]	0/5	2/5 (1.0)[b]	5/5 (1.0)	--
		Corn oil	0/5	--	0/5	5/5 (2.0)	5/5 (2.0)
Kidney	Nephropathy	α-CD	0/5	0/5	5/5 (1.2)	5/5 (3.0)	--
		Corn oil	0/5	--	5/5 (1.0)	5/5 (2.8)	5/5 (3.0)
Adrenal cortex	Cytoplasmic vacuolation	α-CD	0/5	0/5	0/5	5/5 (1.0)	--
		Corn oil	0/5	--	0/5	4/5 (1.0)	5/5 (2.0)
Females							
Liver	Hepatocyte hypertrophy	α-CD	0/5	0/5	0/5	3/5 (1.0)	--
		Corn oil	0/5	--	0/5	5/5 (1.0)	5/5 (2.0)
Adrenal cortex	Cytoplasmic vacuolation	α-CD	0/5	0/5	0/5	2/5 (1.0)	--
		Corn oil	0/5	--	0/5	2/5 (1.0)	4/5 (1.0)

[a]Incidence (number with lesion/number examined)
[b]Average severity is reported in parenthesis: 1.0 = minimal; 2.0 = mild; 3.0 = moderate; 4.0 = marked

oil groups) and 1000 mg/kg-day group (only corn oil tested). Serum cholesterol levels were statistically significantly increased in male and female mice (α-CD vehicle) at 400 mg/kg-day (26 and 36% higher than controls), and male and female mice (corn oil vehicle) at 1000 mg/kg-day (84 and 65% higher). Serum 5'-nucleotidase activities were increased in female mice (α-CD vehicle) at 400 mg/kg-day (30% higher than controls) and male and female mice (corn oil vehicle) at 1000 mg/kg-day (166 and 159% higher than controls). Serum triglyceride levels were increased in female mice (α-CD vehicle) at 50 and 400 mg/kg-day (47 and 53% higher than controls), and female mice (corn oil vehicle) at 1000 mg/kg-day (78% higher than controls). The only organ weight change of note was a 71% increase in liver weight in males and 55% increase in females in the 1000 mg/kg-day corn oil group. No treatment-related gross lesions were observed in males or females of any treatment group. The only microscopic change observed in mice was hepatocellular hypertrophy, characterized by enlargement of the hepatocytes in the centrilobular area, which was observed at $\geq$400 mg/kg-day in males and females (both vehicles). All treated males at $\geq$400 mg/kg-day had this lesion. The incidence in females was 1/5 (α-CD) and 4/5 (corn oil) at 400 mg/kg-day and 5/5 (corn oil) at 1000 mg/kg-day. Severity increased from minimal to mild as the dose increased from 400 mg/kg-day to 1000 mg/kg-day, and was generally greater in males than in females. The hepatocellular hypertrophy is not considered to be adverse because it is likely an adaptive response to chemical exposure. On the basis of serum chemistry changes suggestive of cholestatic liver disease, this study identified a LOAEL of 400 mg/kg-day and NOAEL of 50 mg/kg-day for mice.

Macri et al. (1987) administered 4-chlorobenzotrifluoride (99.3% pure) in olive oil to Sprague-Dawley rats (6 per sex and dose) via oral gavage daily for 28 days at 0, 10, 100 or 1000 mg/kg. Clinical signs of toxicity were recorded at unspecified intervals. Body weight and food and water consumption were recorded daily throughout the treatment period. Clinical chemistry parameters evaluated included glucose, blood urea nitrogen, total proteins, triglycerides, cholesterol, creatinine, bilirubin, serum electrolytes, alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, lactate dehydrogenase and γ-glutamyl transpeptidase. Hematology parameters evaluated included total erythrocyte and leucocyte counts, hemoglobin and differential leucocyte counts. All rats were sacrificed after 28 days of treatment, the liver, kidneys, spleen, heart and adrenals were weighed. These organs and the brain, thymus, lungs, thyroid, stomach, duodenum, pancreas, testes, ovaries and uterus were microscopically examined.

No animals died prior to scheduled sacrifice (Macri et al., 1987). The only clinical sign noted was an increase in the incidence of salivation during the last week of treatment in both males and females treated with 1000 mg/kg-day. Body weights were similar to controls, except for males treated with 1000 mg/kg-day. In this group, the rate of body weight gain was significantly reduced and the final mean body weight was approximately 10% lower than controls. No effects on food or water consumption were observed. Serum chemistry changes included statistically significant, dose-related increases in serum cholesterol and triglyceride levels in males but not in females at $\geq$100 mg/kg-day. Serum cholesterol levels at 10, 100 and 1000 mg/kg-day were 24, 62 and 75% higher than controls, respectively. Serum triglyceride levels at 10, 100 and 1000 mg/kg-day were 19, 92 and 137% higher than controls, respectively. Relative liver weight was significantly increased ($p<0.01$; not otherwise quantified) in males at $\geq$100 mg/kg-day and females at 1000 mg/kg-day. Relative kidney weight was significantly increased only in males ($\geq$100 mg/kg-day). Other organ weights were similar to controls. No treatment-related gross lesions were observed at any dose. Microscopic lesions were observed in the liver, kidney and adrenal cortex. In the liver, the only reported effects were slight to moderate fatty changes in several treated and control animals (incidences not reported). Due to the lack of relation to dose, the liver lesions were not attributed to 4-chlorobenzotrifluoride, but reportedly could have been related to use of the oil vehicle. Severe hyaline droplet nephrosis, often accompanied by dilated tubules and hypercellular foci, was observed in the proximal convoluted tubules of the kidneys in all (6/6) male rats at 1000 mg/kg-day. Slight hyaline droplet nephrosis with granular material was observed in 4/6 males at 100 mg/kg-day. Females at 1000 mg/kg-day showed occasional, slight-to-moderate presence of intraluminal granular material. In the adrenal cortex, marked fatty change (vacuolation of the cells of the zona fasciculata) was observed in 5/6 males at 1000 mg/kg-day, but was not observed in females at this dose or any animals in the lower dose groups. The authors noted that the adrenal cortex vacuolation could indicate decreased adrenal activity and be consistent with the hypercholesterolemia in the males. As previously discussed, the hyaline droplet nephrosis of the kidney, and other effects induced by this compound in male rats, are consistent with α_{2u}-globulin accumulation and are not considered to be relevant to human health risk assessment. Based on the observed hepatic changes (increases in serum cholesterol and triglyceride levels), this study identified a LOAEL of 100 mg/kg-day and a NOAEL of 10 mg/kg-day in rats.

In a 3-month study (Arthur and Probst, 1983), groups of 15 F344 rats/sex were treated daily by gavage with 4-chlorobenzotrifluoride (97.8% pure) in corn oil at doses of 0, 10, 40, 150 or 500 mg/kg-day. The following parameters were evaluated during the 90-day treatment period: ophthalmology (at study initiation and near study termination); clinical signs of toxicity and mortality (daily); detailed physical examination (weekly); and body weight, food consumption and food utilization (weekly). After the treatment period, selected clinical chemistry (serum glucose, urea nitrogen, creatinine, total bilirubin, alkaline phosphatase and alanine transaminase), hematology (erythrocyte count, hemoglobin, PCV, total and differential leukocyte counts, erythrocyte morphology, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration) and urinalysis (color, clarity, specific gravity, pH, protein, glucose and blood) indices were evaluated. Liver p-nitroanisole o-demethylase activity was determined from five rats per sex. After sacrifice, the adrenals, heart, kidneys, liver, ovaries, prostate, spleen, testes, thyroids and uterus were weighed and organ:body weight ratios were calculated. A complete gross examination was conduced at sacrifice, and a comprehensive set of tissues was microscopically examined.

One male rat at 10 mg/kg-day and two male rats at 500 mg/kg-day died (Arthur and Probst, 1983). No significant dose-related physical or behavioral signs or ophthalmic changes were noted. Body weight gain was slightly, but statistically significantly depressed throughout the study in males treated with 500 mg/kg-day. However, terminal body weight in this group was only 5% lower than controls, and not statistically different. Body weights in other treated groups were similar to controls throughout the study. Food consumption and efficiency of utilization were generally similar to controls. Slight, statistically significant decreases in red blood cell count, hemoglobin and hematocrit were noted in males at 500 mg/kg-day. A minimal decrease in hematocrit was also present in males at 150 mg/kg-day. These effects were not seen in females. Serum chemistry changes of note were significant increases in total bilirubin in both males and females at 500 mg/kg-day (46 and 75% higher than controls, respectively) and slightly elevated levels of serum urea nitrogen in male rats at 150 and 500 mg/kg-day. Alkaline phosphatase was significantly elevated in males at all doses, but without a clear dose-response relationship (41, 46, 46 and 40% higher than controls in low to high dose groups), and in females at 500 mg/kg-day (43% higher than controls). Urinalysis indicated mild proteinuria in males at 500 mg/kg-day and females at $\geq$150 mg/kg-day. Hepatic p-nitroanisole o-demethylase activity was significantly increased at $\geq$40 mg/kg-day in males and at $\geq$150 mg/kg-day in females. Significant, dose-related increases in absolute and relative organ weight were observed for the liver ($\geq$10 mg/kg-day in males and $\geq$40 mg/kg-day in females), kidney ($\geq$150 mg/kg-day in males and females) and adrenals ($\geq$150 mg/kg-day in males and 500 mg/kg-day in females). Relative liver weights in males were 8, 13, 36 and 90% higher than controls at 10, 40, 150 and 500 mg/kg-day, respectively. Relative adrenal weights in males were 3, 8, 17 and 50% higher than controls at 10, 40, 150 and 500 mg/kg-day, respectively.

Treatment-related microscopic changes were observed in the liver and kidneys, but not in the adrenals or other tissues (Arthur and Probst, 1983). In the liver, centrilobular hypertrophy was observed in 15/15 males and 1/15 females at 150 mg/kg-day and in 13/15 males and 15/15 females at 500 mg/kg-day. This change was not observed at $\leq$40 mg/kg-day in either sex. Severity increased from slight to moderate in males and from minimal to slight in females as the

dose increased from 150 to 500 mg/kg-day. Kidney lesions included renal tubular degeneration in one low-dose male rat (10 mg/kg-day) and in all male rats dosed at ≥40 mg/kg-day. The severity of the renal lesions was dose-related, ranging from minimal (decreased cellular height, increased cytoplasmic basophilia, increased hyaline droplet formation) to moderate (increased number of necrotic cortical epithelial cells and prominent hyaline casts in tubules of the outer zone medulla and occasionally in the cortex). Tubular degeneration was not observed in female rats at any dose or in male control rats. A general increase in the amount of colloid present in the thyroid occurred with increasing dose; however, the toxicological significance of this observation is not clear because no morphological changes in the thyroid were observed at any dose. As previously discussed, kidney effects induced by 4-chlorobenzotrifluoride are consistent with α_{2u}-globulin accumulation and are not considered to be relevant to human health risk assessment (U.S. EPA, 1991b). The increases in liver weight (≥10 mg/kg-day), hepatic p-nitroanisole o-demethylase activity (≥40 mg/kg-day) and hepatocellular hypertrophy (≥150 mg/kg-day), in the absence of liver histopathology, are indicative of enzyme induction and are not considered by the EPA to be adverse (U.S. EPA, 2002). The increases in serum alkaline phosphatase (≥10 mg/kg-day) are not considered to be adverse because there was no clear dose-response relationship and the magnitude of the changes is unlikely to be toxicologically significant. Based on increased serum bilirubin in both sexes, this study, the EPA identified a LOAEL of 500 mg/kg-day and NOAEL of 150 mg/kg-day in rats.

EBL (1981) reported results of a reproductive/90-day toxicity study with an *in utero* exposure phase. Groups of 20 Sprague-Dawley rats/sex were treated daily by gavage with 4-chlorobenzotrifluoride (97% pure) in corn oil at doses of 0, 5, 15 or 45 mg/kg. The parental generation was treated for 4 weeks before mating, throughout reproduction and through the weaning of the F_1 generation (total treatment duration of 76 to 83 days). The F_1 generation was culled to10 pups/litter on postnatal day (PND) 14. Twenty F_1 rats were randomly selected from each dose group on PND 21 and given the same doses as the F_0 animals for 90 days post-weaning. Mortality, clinical signs of toxicity, body weight and food consumption were recorded over the treatment period. F_1 animals were sacrificed 90 days after the start of treatment. Prior to sacrifice, blood and urine were collected for hematology, clinical chemistry and urinalysis parameters. Hematology and clinical chemistry parameters were also determined for F_0 animals 2 weeks after study initiation. The clinical chemistry evaluations included the following liver enzymes: serum glutamic-oxaloacetic transaminase (i.e., aspartate aminotransferase), serum glutamic-pyruvic transaminase (i.e., alanine aminotransferase) and serum alkaline phosphatase. After sacrifice, the heart, spleen, kidneys, liver, ovaries and testes were weighed, and organ to body weight ratios were calculated. A complete gross pathology examination was conducted, and a comprehensive set of tissues (including liver and adrenals) was microscopically examined from control and high-dose F_1 animals. Tissues from other dose groups were only microscopically examined if gross lesions were present, and tissues from F_0 animals were not evaluated unless gross lesions were observed. Total number of pups born dead and alive per litter, pup sex, weight, litter weight and pup survival were recorded.

No treatment-related mortalities, clinical signs or effects on weight gain or food consumption were observed in the F_0 or F_1 rats (EBL, 1981). Treatment of rats with 4-chlorobenzotrifluoride had no effect on the number of pups/litter, pup survival or length of

gestation period. No clear treatment-related effects on pup body weight were observed. No significant treatment-related changes in hematology or clinical chemistry were noted in F_0 or F_1 rats. At necropsy, no dose-related gross lesions were observed in the F_0 rats; therefore, histological examinations were not performed on these animals. In the F_1 generation, determination of organ weights revealed a nonsignificant dose-related increase in the mean liver weights and mean liver-to-body weight ratios in both sexes. Histological examinations of major tissues and organs completed on F_1 controls and on rats treated at 45 mg/kg-day did not reveal any treatment-related effects. The NOAEL in this study was 45 mg/kg-day, the highest dose tested. A LOAEL was not achieved.

Inhalation Exposure. Newton et al. (1998) exposed Charles River CD rats (25 per sex and dose) via whole body inhalation to 4-chlorobenzotrifluoride (99% pure) vapor at 0, 10, 51 or 252 ppm (0, 74, 377 or 1860 mg/m^3, respectively) 6 hours/day, 5 days/week, for 13 weeks. Selection of the exposure concentrations was based on results of a 4-week preliminary inhalation study in which 10 rats/sex/concentration were exposed to the test substance via whole body inhalation at 0, 100, 262, 494 or 1044 ppm (0, 740, 1940, 3650 or 7710 mg/m^3, respectively) for 6 hours/day and 5 days/week. Although few details regarding the preliminary study were specified in the publication, the following parameters appear to have been evaluated: clinical signs of toxicity, mortality, hematology, clinical chemistry and macroscopic and microscopic pathology. In the 13-week study, the following parameters were recorded during the exposure period: clinical signs of toxicity (daily), body weight (weekly) and food consumption (weekly). All animals were subjected to weekly detailed physical examinations over the 13-week exposure period. Ophthalmic examinations were conducted prior to study initiation and 1 day prior to sacrifice. Motor activity and a functional observational battery were evaluated in 10 animals per group before the start of exposure, at weeks 4, 8 and 13 of exposure, and after a 13-week recovery period (five animals per group). Hematology (blood count) and clinical chemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, fasting glucose, creatine kinase, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, sodium, potassium, chloride, calcium and inorganic phosphorus) parameters were evaluated after 13 weeks of treatment from 10 animals per group. The brain, adrenals, kidneys, liver, lungs, ovaries and testes (with epididymides) were weighed, a complete gross pathology evaluation was performed and a comprehensive set of tissues was microscopically examined from 10 animals per sex and dose at the end of the 13-week exposure and from five animals per sex and dose after the 13-week recovery period. A comprehensive neuropathology examination was conducted on an additional five animals per sex and dose sacrificed at the end of exposure and on five animals per sex and dose after the 13-week recovery period; tissues other than the brain and spinal cord were not examined from these animals. 4-Chlorobenzotrifluoride levels were determined in the blood and tissues of three female animals at each sacrifice period.

No deaths occurred in the 4-week study (Newton et al., 1998). Dose-related increases in activity were noted in both male and female rats at $\geq$262 ppm that seemed to become more prominent as the daily exposure sessions progressed. In the detailed weekly observations, hyperactivity and tremors were reported, but only at 1044 ppm. Hematology findings were unremarkable. The serum chemistry analysis showed small but statistically significant increases in serum protein in both sexes at 492 and 1044 ppm (5-6 and 11% higher than controls,

respectively). Similar increases in serum calcium and phosphorous occurred at ≥492 ppm, but the effects could be related to the increase in serum protein because these chemicals are bound to proteins in serum. Absolute and relative liver weights were significantly increased in a dose-related fashion in males and females at ≥492 ppm. Absolute and/or relative kidney weights were increased in males and females at ≥262 ppm, but the changes were slight and not directly related to dose. Pathological examinations found dose-related increases in incidence and severity of centrilobular hepatocellular hypertrophy in the liver of male and female rats at ≥100 ppm. Incidence was 7/10 males and 0/10 females, 10/10 males and 5/10 females, 10/10 males and 10/10 females and 10/10 males and 10/10 females in the 100, 262, 492 and 1044 ppm groups, respectively. From the write-up, it appears that the lesion was not found in controls, although control incidence was not specifically reported. Severity increased from minimal at 100 ppm to moderate at the higher doses. Male rats exposed to 262 ppm and above had intracytoplasmic eosinophilic granules in the epithelium lining of the proximal convoluted tubules that were shown to contain α_{2u}-globulin. The LOAEL in this study is 1044 ppm based on clinical signs of neurotoxicity. The NOAEL is 492 ppm because the liver effects are not considered to be adverse; the hepatocellular hypertrophy is likely an adaptive effect reflecting the induction of hepatic cytochrome P-450 (P450) enzymes (see discussion of the 13-week study below), and the increases in serum proteins were small and unlikely to be clinically significant.

In the 13-week study, there were no deaths and no clinical signs were found during the exposures or during the detailed weekly observations (Newton et al., 1998). Body weights were unaffected and there was only a minimal effect on food consumption (6% decrease in the 252 ppm group during the first 2 weeks of the study). The neurobehavioral, neuropathology and hematology evaluations were unremarkable. Clinical chemistry changes at the end of the exposure period included small but statistically significant increases in serum total protein level, albumin level and serum alanine aminotransferase (ALT) activity in females at 252 ppm (15, 19 and 40% higher than controls, respectively). No serum chemistry changes occurred in males at the end of the exposure period or in either sex after the 13-week recovery period. Statistically significant organ weight changes were increased relative liver weights in male and female rats at 252 ppm and increased absolute kidney weight in males at 252 ppm. No gross lesions were observed at any concentration. Histopathology examinations revealed hepatocellular hypertrophy in 3/10 males and 3/10 females at 252 ppm. The highest tested concentration, 252 ppm, is classified as a NOAEL based on liver effects. As discussed below, the increases in hepatocellular hypertrophy and liver weight are likely to be adaptive responses to 4-chlorobenzotrifluoride exposure, and the serum chemistry findings are insufficient evidence of liver damage.

Experimental data support the speculation that the hepatocellular hypertrophy and increased liver weight are due to induction of hepatic P450. Pelosi et al. (1998) found that the rats exposed to 252 ppm 4-chlorobenzotrifluoride for 13 weeks in the Newton et al. (1998) study had slight, but statistically significant, increased activities of various P450 enzymes. Although hepatic P450 isozymes were not evaluated after 4 weeks of exposure, the finding that the 13-week exposure did not dramatically increase P450 activities is consistent with the diminished incidences of hepatocellular hypertrophy after 13 weeks. Incidences of hepatocellular hypertrophy decreased with continued exposure; for example, in the male rats, the incidences

were high after 4 weeks (7/10-10/10 at 100-1044 ppm) and only minimally increased after 13 weeks (3/10 at 252 ppm). No hepatocellular hypertrophy was observed after the 13-week recovery period. Other evidence provides no indication that the hepatocellular hypertrophy and increased liver weight were part of a spectrum of liver toxicity. The increases in relative liver weight were small and did not increase with duration of exposure (10-11% in both sexes at 262 ppm after 4 weeks and at 252 ppm after 13 weeks). The increases in serum ALT, total protein and albumin at 252 ppm after 13 weeks also were small, of questionable toxicological significance, only occurred in females (the less sensitive sex based on the hypertrophy findings in the 4-week study), and were not accompanied by increases in other liver enzymes (serum AST and AP) in either sex. Additionally, the only histological change in the liver was hepatocellular hypertrophy; no degenerative or other kinds of adverse lesions were induced.

A PBPK model for inhaled 4-chlorobenzotrifluoride was developed to facilitate extrapolation of the results of the Newton et al. (1998) rat inhalation toxicity studies to humans (Knaak et al., 1998). Concentrations of 4-chlorobenzotrifluoride in human tissues were estimated using a model that was derived from human and rat partition coefficients, scaled metabolic rate constants and default human physiological characteristics. The blood:air partition coefficient was found to be a primary determinant of the fate of inhaled 4-chlorobenzotrifluoride; percentage of body fat and muscle were also important. Simulations using the model predicted similar tissue doses (area under the curve or AUC) in the livers of rats and humans exposed to 4-chlorobenzotrifluoride under the same conditions (10, 50 or 250 ppm for 1 day or 13 weeks).

A study from the Russian literature (Rapoport et al., 1986) included exposure of male nonpurebred albino rats (numbers not specified) to 4-chlorobenzotrifluoride at 5.5, 20.5, 71.6 or 440 mg/m^3 continuously for 120 hours, with subsequent observation for at least 115 days. Examinations included body weight, serum chemistry and hematology and neurobehavior ("summation-threshold index," motor activity, grasping reflex). Exposure to 440 or 71.6 mg/m^3 resulted in a change in "practically all of the parameters studied." The 20.5 mg/m^3 concentration was considered the "minimally effective" concentration, while the 5.5 mg/m^3 was considered to be "subthreshold." The results are presented as time of onset of significant changes in parameters, which generally increased with decreasing exposure concentration. The study does not relate exposure concentration to incidence or severity of effects. Therefore, a reliable NOAEL or LOAEL cannot be derived from this study.

Other Studies

Genotoxicity testing results for 4-chlorobenzotrifluoride are mostly negative. 4-Chlorobenzotrifluoride did not induce reverse mutations in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 with or without addition of exogenous metabolic activation (S9) (NTP, 1992; Haworth et al., 1983; Litton Bionetics, 1978a; Mazza et al., 1986) or gene conversions in *Saccharomyces cerevisiae* strain D4, +/- S9 (Litton Bionetics, 1978a). The substance was also negative in a DNA repair deficiency assay with *Escherichia coli* W3110 polA+ and P3478 polA- tester strains, +/- S9 (Litton Bionetics, 1978b). Urine from mice treated with the substance also tested negative in *S. typhimurium* TA1535, TA1537, TA98 and TA100 (Litton Bionetics, 1979a). The substance did not induce forward mutations at the thymidine

kinase (TK) locus in mouse lymphoma L5178Y cells, +/- S9 (Litton Bionetics, 1978c). 4-Chlorobenzotrifluoride induced sister chromatid exchanges (SCEs) in mouse lymphoma L5178Y cells, +/- S9 (Litton Bionetics, 1979b), but did not induce chromosomal aberrations in Chinese hamster ovary cells, +/- S9 (Lilly Research Laboratories, 1983a). Negative results were also reported for 4-chlorobenzotrifluoride in an *in vivo* chromosomal aberration assay in rats (Lilly Research Laboratories, 1983b). The substance was positive in an assay for unscheduled DNA synthesis in EUE cells (not further described) (Benigni and Dogliotti, 1980). Litton Bionetics (1980) reported that 4-chlorobenzotrifluoride tested negative for cell transformation in Balb/C3T3 cells; the study did not report whether an activating system was used. Lilly Research Laboratories (1983c) also found that 4-chlorobenzotrifluoride (97% pure) tested negative for cell transformation in Balb/C3T3 cells in the presence of S9.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR 4-CHLOROBENZOTRIFLUORIDE

The available animal data indicate that the liver, kidneys and adrenals are the most sensitive targets of response for 4-chlorobenzotrifluoride after oral exposure. Liver effects included increases in organ weight, hepatocellular hypertrophy and serum chemistry changes (increases in cholesterol, triglycerides, 5'-nucleotidase, bile acids and bilirubin) in rats and mice (NTP, 1992; Macri et al., 1987; Arthur and Probst, 1983). Adrenal effects included increased organ weight and cytoplasmic vacuolation in the cortex in rats (NTP, 1992; Macri et al., 1987). Renal effects included increased organ weight, hyaline droplet accumulation, tubule dilation, inflammation of the interstitial cells and regeneration of the epithelium in male rats (NTP, 1992; Macri et al., 1987; Arthur and Probst, 1983). The effects on the kidneys appear to be related to α_{2u}-globulin accumulation, a male rat-specific effect that is not predictive for health effects in humans (U.S. EPA, 1991b). Support for this relationship comes from the observation that kidney lesions were observed only in male rats in these studies, and not female rats or mice of either sex; the types of lesions reported are consistent with those typical of α_{2u}-globulin accumulation, as described in U.S. EPA (1991b); and α_{2u}-globulin levels in the kidney were significantly increased and correlated with the presence of renal lesions in male rats in the NTP (1992) study. Other effects of 4-chlorobenzotrifluoride included minor hematological alterations at doses in the range of those causing the hepatic serum chemistry and adrenal effects (NTP, 1992; Arthur and Probst, 1983). A limited one-generation reproductive toxicity assessment found no effects on reproductive function in rats (EBL, 1981), suggesting that the reproductive system is not a sensitive target for 4-chlorobenzotrifluoride. No developmental toxicity studies on 4-chlorobenzotrifluoride were located; therefore, it is not possible to determine whether the chemical is a developmental toxicant.

Hepatocellular hypertrophy occurred at doses as low as 50 mg/kg-day (NTP, 1992) and 150 mg/kg-day (Arthur and Probst, 1983) in rats and 400 mg/kg-day in mice (NTP, 1992). However, this effect alone is not considered by the EPA to be adverse because, in the absence of other indicators of liver toxicity, such as histopathology or changes in serum chemistry, it likely reflects adaptive enzyme induction. The serum chemistry changes observed in rats and mice are indicative of cholestasis (an impaired production or flow of bile) and therefore are considered

indicators of liver toxicity. Increases in serum bilirubin, alkaline phosphatase, gamma glutamyl transpeptidase, 5'-nucleotidase, cholesterol, triglycerides and total bile acids are associated with cholestasis (U.S. EPA, 2002; NTP, 1992) and serum 5'-nucleotidase activity is a particularly sensitive and specific indicator of biliary duct damage in rats, mice and humans (Carakostas et al., 1986, 1990). NTP (1992) suggested a cellular rather than physical mechanism for 4-chlorobenzotrifluoride cholestasis because the serum chemistry changes were not accompanied by histopathologic evidence of hepatocellular toxicity. Cellular mechanisms that could account for the cholestatic effects include 4-chlorobenzotrifluoride-induced alterations in plasma membranes (function and composition), disruptions in microfilaments and microtubules, loss of tight junction integrity and/or changes in the concentration and composition of bile acids (NTP, 1992). U.S. EPA (2002) similarly indicates that cholestasis can be due to hepatocellular dysfunction that is not evident histologically. The lowest dose of 4-chlorobenzotrifluoride that induced a definitive serum chemistry change was 100 mg/kg-day for increased cholesterol and triglycerides in male rats (62 and 92% higher than controls, respectively) (Macri et al., 1987). Changes in these indices were generally dose-related, also occurring at 400 and 1000 mg/kg-day in one or both sexes; at 1000 mg/kg-day, serum cholesterol was increased 75-134% in rats and 65-84% in mice (NTP, 1992; Macri et al., 1987) and serum triglyceride was increased 71-137% in rats and 78% in mice (NTP, 1992; Macri et al., 1987). Other serum chemistry changes at doses above 100 mg/kg-day included increases in 5'-nucleotidase at 400 and 1000 mg/kg-day in rats (13-21% and 46-62%) and mice (30% and 159-166%) in one or both sexes (NTP, 1992), serum bilirubin at 500 mg/kg-day in male and female rats (46-75%) (Arthur and Probst, 1983) and total bile acids at 1000 mg/kg-day in male rats (123%) (NTP, 1992). Increases in serum alkaline phosphatase were observed at doses ranging from 10-500 mg/kg-day in rats (Arthur and Probst, 1983), but the response was not dose-related or of magnitudes likely to be toxicologically significant; additionally, serum alkaline phosphatase was not increased in rats exposed to $\leq$45 mg/kg-day (EBL, 1981).

Adrenal cortex cytoplasmic vacuolation occurred in male and female rats exposed to 400 and 1000 mg/kg-day for 2 weeks (NTP, 1992) and males rats exposed to 1000 mg/kg-day for 4 weeks (Macri et al., 1987). No histological changes occurred in the adrenals of male or female rats exposed to $\leq$500 mg/kg-day for 3 months (Arthur and Probst, 1983) or $\leq$45 mg/kg-day for 3 months (EBL, 1981). NTP (1992) concluded that the cause and biological significance of the adrenal lesion are not clear because vacuolation and other microscopic changes are often observed in the adrenal cortex in toxicity studies, and the study was not designed for determining if it was due to stress, an increase in ACTH stimulation due to impaired adrenal steroid production or other mechanisms. Macri et al. (1987) observed that the effect could indicate decreased adrenal activity and be consistent with the observed hypercholesterolemia.

The primary liver and adrenal findings (NTP, 1992; Macri et al., 1987), summarized in Table 2, are that male rats are the species and sex that appear to be most sensitive to 4-chlorobenzotrifluoride. The lowest dose of 4-chlorobenzotrifluoride that induced serum chemistry changes likely to be toxicologically significant was 100 mg/kg-day for increased cholesterol and triglycerides in rats exposed for 28 days (Macri et al., 1987), indicating that this dose is the LOAEL for subchronic oral exposure based on liver effects; the NOAEL is 50 mg/kg-day (NTP, 1992). The lowest dose inducing adrenal cortex cytoplasmic vacuolation, a possible

Table 2. Effects of Oral Exposure to 4-Chlorobenzotrifluorideon Selected Liver and Adrenal Endpoints in Male Rats

Effect	Dose (mg/kg-day)									Reference
	0	10	40	50	100	150	400	500	1000	
Hepatocellular hypertrophy[f]	0/5			0/5			5/5		5/5	NTP, 1992[a,c]
	0/5	0/5		2/5			5/5			NTP, 1992[b,c]
	0/6	0/6			0/6				0/6	Macri et al., 1987[d]
	0/15	0/15	0/15			15/15		13/15		Arthur and Probst, 1983[e]
Serum cholesterol (% increase)	-			8			51[g]		134[g]	NTP, 1992[a]
	-	3		17[g]			37[g]			NTP, 1992[b]
	-	24			62[g]				75[g]	Macri et al., 1987
	not evaluated									Arthur and Probst, 1983
Serum triglycerides (% increase)	-			10			17		54	NTP, 1992[a]
	-	3		(-8)			(-18)			NTP, 1992[b]
	-	19			92[g]				137[g]	Macri et al., 1987
	not evaluated									Arthur and Probst, 1983
Serum 5'-nucleotidase (% increase)	-			2			13[g]		62[g]	NTP, 1992[a]
	-	4.3		10[g]			15[g]			NTP, 1992[b]
	not evaluated									Macri et al., 1987
	not evaluated									Arthur and Probst, 1983
Serum total bile acids (% increase)	-			0			9		123[g]	NTP, 1992[a]
	-	3.4		(-20)			(-16)			NTP, 1992[b]
	not evaluated									Macri et al., 1987
	not evaluated									Arthur and Probst, 1983
Serum bilirubin (% increase)	-	0[f]			0[f]				0[f]	Macri et al., 1987
	-	4	(-6)			23		46[g]		Arthur and Probst, 1983
	not evaluated									NTP, 1992
Serum alkaline phosphatase (% increase)	-	41[g]	46[g]			46[g]		40[g]		Arthur and Probst, 1983
	not evaluated									NTP, 1992
	not evaluated									Macri et al., 1987
Adrenal cortex cytoplasmic vacuolation[f]	0/5			0/5			4/5		5/5	NTP, 1992[a]
		0/5		0/5			5/5			NTP, 1992[b]
	0/6	0/6			0/6				5/6	Macri et al., 1987
	0/15[h]	0/15[h]	0/15[h]			0/15[h]		0/15[h]		Arthur and Probst, 1983

[a]Corn oil vehicle
[b]α-Cyclodextrin vehicle
[c]14-Day study, n=5 per group
[d]28-Day study, n=6 per group
[e]90-day study, n=15 per group
[f]Incidence data not statistically evaluated in any study
[g]Statistically different from control group
[h]No histological effects in adrenals (incidence data not specifically reported)

adverse effect, is 400 mg/kg-day (NTP, 1992), which is higher than the 100 mg/kg-day LOAEL based on liver effects. Key effect levels in the available oral studies of 4-chlorobenzotrifluoride are summarized in Table 3.

The Macri et al. (1987) study was selected as the key study for derivation of the RfD. The critical endpoint(s) chosen for analysis from this study were changes in serum cholesterol and serum triglycerides observed in rats. Based on the database available, this study identifies the lowest subchronic LOAEL for 4-chlorobenzotrifluoride, 100 mg/kg-day for hepatic effects, and provides the most appropriate basis for benchmark dose modeling for derivation of a reference value. The study was conducted over a range of exposure concentrations, included a control group, and demonstrated a dose-related effect. These changes in serum chemistry are indicators of liver toxicity associated hepatocellular hypertrophy. Available continuous-variable models in the EPA Benchmark Dose Software (linear, polynomial, power and Hill models; BMDS version 1.3.2) were fit to the dose-response data for changes in serum cholesterol and triglycerides in male rats. The BMDs and 95% lower confidence limits (BMDLs) calculated for these endpoints are estimates of the doses associated with a change of 1 standard deviation from the control (U.S. EPA, 2000). The predicted BMDs and BMDLs for the two endpoints are summarized in Table 4 and detailed in Appendix A.

Table 3. Summary of Effect Levels from Oral Studies of 4-Chlorobenzotrifluoride

Study	Exposure Duration	Species (Strain)	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Main Endpoints
Macri et al. (1987)	4 weeks	rat (SD)	10	100	increased serum cholesterol and triglycerides
NTP (1992)	2 weeks	rat (F344)	50	400	increased serum cholesterol, adrenal cortex vacuolation
NTP (1992)	2 weeks	mouse (B6C3F1)	50	400	increased serum cholesterol and triglycerides
Arthur and Probst (1983)	3 months	rat (F344)	150	500	increased serum bilirubin
EBL (1981)	3 months	rat (SD)	45	ND	none

SD= Sprague-Dawley; ND = not determined

Table 4. Summary of BMD Model Predictions for Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days[a]

Endpoint	$BMD_{1\ SD}$ (mg/kg-day)	$BMDL_{1\ SD}$ (mg/kg-day)
Serum cholesterol level	NA	NA
Serum triglycerides level	15.1	8.8

[a]Doses associated with a 1 standard deviation change from the control
NA = not available: no adequate fits were obtained

The $BMDL_{1\ SD}$ of 8.8 mg/kg-day derived by benchmark dose modeling of the Macri et al. (1987) serum triglycerides data is similar to the NOAEL of 10 mg/kg-day for increases in serum triglycerides and cholesterol in this study. Because the $BMDL_{1\ SD}$ incorporates more information about the shape of the dose-response curve than the NOAEL, the $BMDL_{1\ SD}$ is used as the point of departure for derivation of the RfD. The $BMDL_{1\ SD}$ of 8.8 mg/kg-day is divided by a composite uncertainty factor (UF) of 300 [10 for interspecies extrapolation, 10 for human variability and 3* for database deficiencies (discussed below)] to derive a provisional **subchronic RfD of 3E-2 mg/kg-day**, as follows:

$$\begin{aligned} sRfD &= BMDL_{1\ SD} / UF \\ &= 8.8 \text{ mg/kg-day} / 300 \\ &= 0.03 \text{ or } 3E\text{-}2 \text{ mg/kg-day} \end{aligned}$$

*Half-log of 10 rounded to 3

Because no chronic oral toxicity studies were located in the literature, an additional UF of 10 is applied to the provisional subchronic RfD to derive the provisional **chronic RfD of 3E-3** mg/kg-day, as follows:

$$\begin{aligned} RfD &= sRfD \div UF \\ &= 0.03 \text{ mg/kg-day} \div 10 \\ &= 0.003 \text{ or } 3E\text{-}3 \text{ mg/kg-day} \end{aligned}$$

Confidence in the key study is medium. The study evaluated a sufficient number of doses and an adequate set of toxicological parameters in rats, but is limited by small numbers of animals (6/sex/dose) and a duration of 28 days. The key study is supported by 14-day studies in rats and mice that produced similar changes in serum chemistry indicative of cholestatic liver damage (NTP, 1992). Confidence in the database is medium to low. The database lacks a comprehensive study longer than 28 days in duration; a 90-day study in rats provides limited support for the key study because its serum chemistry evaluations only included a few of the endpoints assessed in the key study. The database is also limited by the absence of developmental toxicity studies. Reproductive toxicity was examined but only in a single-generation study that included assessment of few reproductive endpoints and did not include doses high enough to produce adverse effects on any endpoint (EBL, 1981). Confidence in the provisional subchronic RfD is medium. Confidence in the chronic RfD is low because no chronic toxicity studies were located.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR 4-CHLOROBENZOTRIFLUORIDE

Only one adequately conducted inhalation toxicity study was located (Newton et al., 1998). This study included 4- and 13-week experiments in rats, each of which included evaluations of a comprehensive set of standard toxicological parameters. Both experiments identified the liver as the most sensitive target of response for 4-chlorobenzotrifluoride. Liver

effects in the two experiments included hepatocellular hypertrophy, increased liver weight and minor changes in serum chemistry (small increases in serum protein and, in the 13-week experiment, a small increase in serum ALT). As discussed previously, the liver effects are not considered to be adverse because (1) the hepatocellular hypertrophy and increased liver weight are likely adaptive responses reflecting the induction of hepatic P450 enzymes, (2) the serum chemistry changes were small and of questionable toxicological significance and (3) there is no histological evidence (adverse lesions) to suggest that the hypertrophy, increased liver weight and serum chemistry changes are part of a spectrum of liver toxicity. Other effects included clinical signs of neurotoxicity (hyperactivity and tremors) at the highest exposure level in the 4-week study. The kidney also was identified as a target in male rats in the 4-week study; however, these effects were associated with α_{2u}-globulin accumulation and are not considered relevant to human health risk assessment (U.S. EPA, 1991b). The 4-week study identified a NOAEL of 492 ppm based on the liver effects and a LOAEL of 1044 ppm based on the clinical signs of neurotoxicity. The EPA identified a NOAEL of 252 ppm for liver effects from the 13-week study; this was the highest tested concentration, precluding identification of a possible LOAEL. The 13-week NOAEL of 252 ppm was chosen, based on length of the study, as the most appropriate point of departure for derivation of the RfC.

Calculation of an RfC using the 252 ppm NOAEL in rats first involves determination of a human equivalent concentration (HEC). The U.S. EPA (1994b) default procedure for calculating the HEC for an extrarespiratory effect from a vapor is to adjust for intermittent exposure and multiply the duration-adjusted NOAEL by the ratio of animal to human blood:air partition coefficients, as follows:

$NOAEL_{mg/m^3}$ = ppm x MW / 24.45
= 252 x 180.55 / 24.45
= 1860.9

$NOAEL_{ADJ}$ = 1860.9 mg/m^3 x 6 hours/24 hours x 5 days/7 days
= 332 mg/m^3

$NOAEL_{HEC}$ = $NOAEL_{ADJ}$ x $(H_{b/g})_A / (H_{b/g})_H$
= 332 mg/m^3 x 1
= 332 mg/m^3

where,

$(H_{b/g})_A / (H_{b/g})_H$ = rat to human blood:air partition coefficient ratio
= default ratio of 1, because L_R (47.9; Knaak et al., 1998) is greater than L_H (16.7; Knaak et al., 1998)

The PBPK model developed by Knaak et al. (1998) supports this derivation of human equivalent concentration. The blood:air partition coefficient was found to be a primary determinant of 4-chlorobenzotrifluoride disposition in the model, and that is the factor used to make the adjustment to human equivalent concentration in the default EPA procedure. The

results of the PBPK modeling simulations performed by Knaak et al. (1998) show delivery of similar doses to the liver in humans and rats with intermittent subchronic exposure to 250 ppm, a concentration virtually the same as the NOAEL of 252 ppm, indicating that a multiplier of 1 is appropriate to calculate $NOAEL_{HEC}$ from $NOAEL_{ADJ}$.

The $NOAEL_{HEC}$ of 332 mg/m^3 is divided by a composite UF of 100 (10 for human variability, 3* for toxicodynamic considerations of interspecies extrapolation after making the toxicokinetic adjustments described above and 3* for database deficiencies, including lack of a subchronic LOAEL in rats exposed for longer than 4 weeks, lack of subchronic study in a second species and lack of reproductive and developmental toxicity studies) to derive a provisional **subchronic RfC of 3E-0 mg/m^3**, as follows:

$$\begin{aligned} sRfC &= NOAEL_{HEC} / UF \\ &= 332 \text{ mg/m}^3 / 100 \\ &= 3 \text{ or } 3E\text{-}0 \text{ mg/m}^3 \end{aligned}$$

*Half-log of 10 rounded to 3

Because no chronic inhalation toxicity studies were located in the literature, an additional UF of 10 is applied to the provisional subchronic RfC to derive a provisional **chronic RfC of 3E-1** mg/m^3, as follows:

$$\begin{aligned} RfC &= sRfC \div UF \\ &= 3 \text{ mg/m}^3 \div 10 \\ &= 0.3 \text{ or } 3E\text{-}1 \text{ mg/m}^3 \end{aligned}$$

Confidence in the key study is high. The study evaluated a sufficient number of doses and an adequate set of toxicological parameters. The study design also included a comprehensive neurotoxicology evaluation. Confidence in the database is low. No other inhalation toxicity studies are available that support the key study. In addition, the database is limited by the absence of adequate reproductive and developmental toxicity studies. The results of a one-generation oral study (EBL, 1981) suggest that the reproductive system is not likely to be a sensitive target for 4-chlorobenzotrifluoride, but only a limited evaluation of reproductive toxicity endpoints was performed. Based on the limited inhalation toxicity database, low confidence in the provisional subchronic and chronic RfC follows.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 4-CHLOROBENZOTRIFLUORIDE

Relevant studies regarding the carcinogenicity of 4-chlorobenzotrifluoride in humans or animals following oral or inhalation exposure were not located. Available genotoxicity data indicate that 4-chlorobenzotrifluoride is not a potent genotoxic agent. In accordance with current EPA cancer guidelines (U.S. EPA, 2005), the available data are inadequate for an assessment of human carcinogenic potential. Therefore, derivation of quantitative estimates

(oral slope factor or inhalation unit risk) of cancer risk for 4-chlorobenzotrifluoride is precluded by the absence of carcinogenicity data.

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APPENDIX A. DETAILS OF BMD ANALYSIS FOR THE SUBCHRONIC ORAL RfD FOR 4-CHLOROBENZOTRIFLUORIDE

Hepatic effects data from the Macri et al. (1987) 28-day study in rats were used for benchmark dose modeling. Available continuous-variable models in the EPA Benchmark Dose Software (linear, polynomial, power and Hill models; BMDS version 1.3.2) were fit to the data for changes in serum cholesterol and triglycerides in male rats shown in Table A-1. The BMDs and 95% lower confidence limits (BMDLs) calculated for these endpoints are estimates of the doses associated with a change of 1 standard deviation from the control (U.S. EPA, 2000).

Table A-1. Serum Levels of Cholesterol and Triglycerides in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days (Macri et al., 1987)

Dose (mg/kg-day)	0	10	100	1000
Group size	6	6	6	6
Serum cholesterol (mg/100 mL)[a]	56.50 ± 7.50	70.17 ± 8.97	91.33 ± 23.69[b]	98.83 ± 34.69[c]
Serum triglycerides (mg/100 mL)[a]	33.50 ± 6.05	39.83 ± 7.82	64.33 ± 30.30[b]	79.33 ± 25.28[c]

[a]Values are means ± standard deviation
[b]Statistically significant increase from control value ($p<0.05$)
[c]Statistically significant increase from control value ($p<0.01$)

The simplest model (linear) was applied to the data first while assuming constant variance. If the data were consistent with the assumption of constant variance ($p\geq0.05$), then the fit of the linear model to the means was evaluated. If the linear model adequately fit the means ($p\geq0.1$), then it was selected as the model for BMD derivation. If the linear model did not adequately fit the means, then the more complex models were fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p\geq0.1$), the one with the lowest AIC for the fitted model was selected for BMD derivation. If the test for constant variance was negative, the linear model was run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provided an adequate fit ($p\geq0.05$) to the variance data, then the fit of the linear model to the means was evaluated. If the linear model did not provide adequate fit to the means while the variance model was applied, then the polynomial, power, and Hill models were fit to the data and evaluated while the variance model was applied. Among those providing adequate fit to the means ($p\geq0.1$), the one with the lowest AIC for the fitted model was selected for BMD derivation. If the test for constant variance was negative and the nonhomogenous variance model did not provide an adequate fit to the variance data, then the data set was considered not to be suitable for BMD modeling. The predicted BMDs and BMDLs for the two endpoints are detailed below.

SERUM CHOLESTEROL DATA

For the serum cholesterol data, the assumption of constant variance did not hold. The nonhomogeneous variance model was applied and provided adequate fit to the variance. With the nonhomogeneous variance model applied, the linear model did not provide an adequate fit to the means, and neither did any of the other available models (insufficient degrees of freedom for Hill model). Results are shown in Table A-2. Given that the most pertinent part of the dose-response curve is that which lies at the lower doses, the high-dose data point (1000 mg/kg-day) was removed from the dataset and the model fitting was conducted, as described before, in an attempt to achieve an adequate fit of the models to the data. As with the complete data set, the assumption of constant variance did not hold, but the nonhomogeneous variance model provided adequate fit to the variance. Sufficient degrees of freedom were available for fitting only the linear model. However, the fit of the linear model to the means was not adequate. Results are shown in Table A-3.

Table A-2. Model Predictions for Changes in Serum Cholesterol Levels in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days

Model	Variance p-value[a]	Means p-value[a]	AIC	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear (constant variance)	0.0007	0.0339	178.33	775.61	465.39
Linear (modeled variance)	0.5294	<0.0001	178.71	494.25	202.50
Polynomial[b,c] (modeled variance)	0.5294	<0.0001	178.71	494.47	202.50
Power[d] (modeled variance)	<0.0001	<0.0001	180.71	495.23	202.50
Hill[d] (modeled variance)	0.5294	NA	161.64	4.64	NA

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]2-degree polynomial; no adequate fit with any polydegree.
[c]betas restricted to ≥ 0.
[d]power restricted to ≥ 1.

AIC = Akaike's Information Criteria; *p* = *p* value from the Chi-squared test; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output).

Table A-3. Model Predictions for Changes in Serum Cholesterol Levels in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days (High Dose Dropped)

Model	Variance p-value[a]	Means p-value[a]	AIC	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear (constant variance)	0.0082	0.1945	118.49	48.07	32.03
Linear (modeled variance)	0.3584	0.04357	116.12	25.12	12.90
Polynomial[b,c] (modeled variance)	0.3584	NA	116.04	26.34	13.05
Power[d] (modeled variance)	0.0071	NA	117.98	25.32	13.14
Hill[d] (modeled variance)	NA				

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]2-degree polynomial; no adequate fit with any polydegree.
[c]betas restricted to ≥ 0.
[d]power restricted to ≥ 1.

AIC = Akaike's Information Criteria; *p* = *p* value from the Chi-squared test; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output).

SERUM TRIGLYCERIDE DATA

For the serum triglyceride level data, the assumption of constant variance did not hold. The nonhomogeneous variance model was applied and provided adequate fit to the variance. With the nonhomogeneous variance model applied, the linear model did not provide an adequate fit to the means, and neither did any of the other available models (insufficient degrees of freedom for Hill model). Results are shown in Table A-4. The results after dropping the high dose are shown in Table A-5. As with the complete data set, the assumption of constant variance did not hold, but the nonhomogeneous variance model provided adequate fit to the variance. Sufficient degrees of freedom were available for fitting only the linear model. The linear model provided adequate fit to the means while the variance model was applied, resulting in a predicted $BMD_{1\ SD}$ and $BMDL_{1\ SD}$ of 15.1 and 8.8 mg/kg-day, respectively (Table A-5, Figure A-1).

Table A-4. Model Predictions for Changes in Serum Triglyceride Levels in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days

Model	Variance p-value[a]	Means p-value[a]	AIC	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear (constant variance)	0.0003	0.0440	174.48	572.29	376.90
Linear (modeled variance)	0.5716	<.0001	177.82	486.21	237.10
Polynomial[b,c] (modeled variance)	0.5716	<.0001	177.82	486.21	237.10
Power[d] (modeled variance)	0.3041	<.00001	179.82	486.21	237.10
Hill[d] (modeled variance)	0.5719	NA	158.71	9.36	NA

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]2-degree polynomial; no adequate fit with any polydegree.
[c]betas restricted to ≥ 0.
[d]power restricted to ≥ 1.

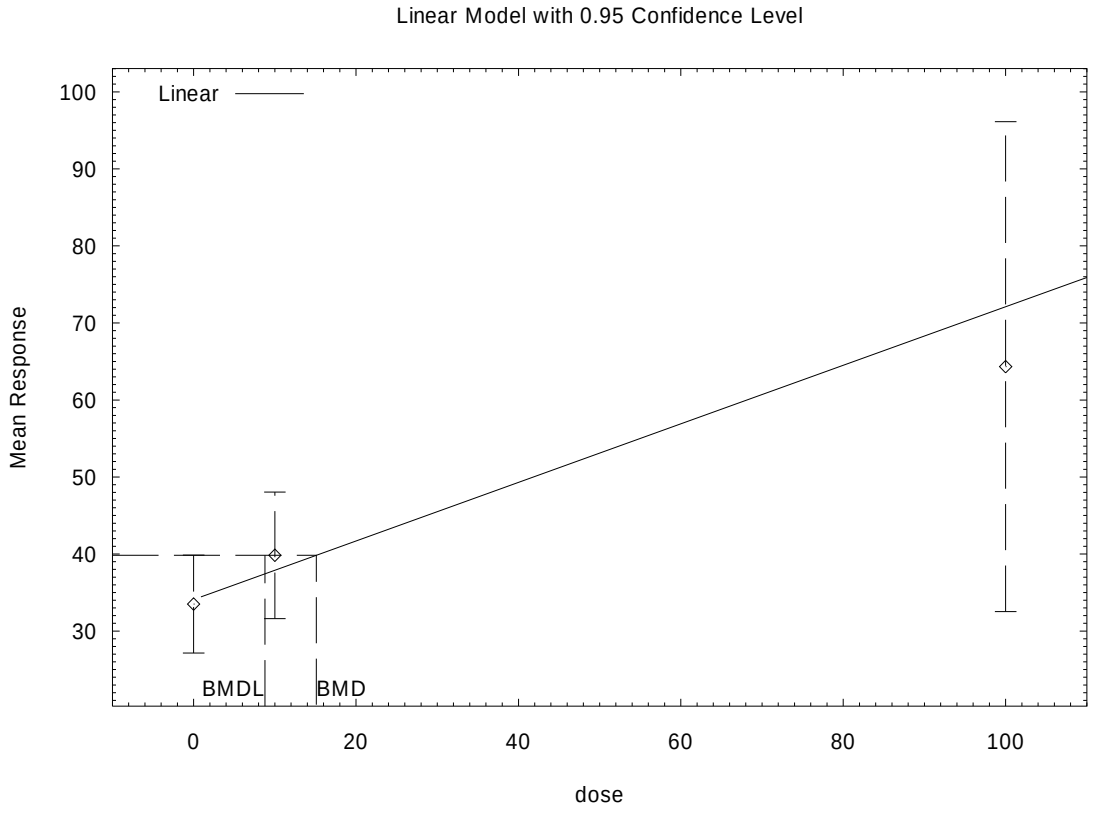
AIC = Akaike's Information Criteria; *p* = *p* value from the Chi-squared test; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output).

Table A-5. Model Predictions for Changes in Serum Triglyceride Levels in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days (High Dose Dropped)

Model	Variance p-value[a]	Means p-value[a]	AIC	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear (constant variance)	0.0001	0.7261	123.69	57.33	36.56
Linear (modeled variance)	0.6451	0.3826	110.91	15.11	8.78
Polynomial[b,c] (modeled variance)	0.6451	NA	113.20	17.37	8.49
Power[d] (modeled variance)	0.4062	NA	112.69	17.72	9.00
Hill[d] (modeled variance)	NA				

[a]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[b]2-degree polynomial; no adequate fit with any polydegree.
[c]betas restricted to ≥ 0.
[d]power restricted to ≥ 1.

AIC = Akaike's Information Criteria; *p* = *p* value from the Chi-squared test; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output).



[a]BMDs and BMDLs indicated are associated with a change of 1 standard deviation from the control, and are in units of mg/kg-day.

Figure A-1. Changes in Serum Triglyceride Levels in Male Rats Exposed to 4-Chlorobenzotrifluoride by Daily Gavage for 28 Days (High Dose Dropped)