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Provisional Peer Reviewed Toxicity Values for

Chlorobenzene
(CASRN 108-90-7)

Superfund Health Risk Technical Support Center
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Cincinnati, OH 45268

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 CHLOROBENZENE (CASRN 108-90-7)

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 science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. 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

IRIS (U.S. EPA, 2006) lists an RfD of 2E-2 mg/kg-day for chlorobenzene based on a NOAEL of 27 mg/kg-day (adjusted dose of 19.6 mg/kg-day) and LOAEL of 54.5 mg/kg-day (adjusted dose of 39.3 mg/kg-day) for liver histopathology in dogs given gelatin capsules containing chlorobenzene for 13 weeks (Hazleton Laboratories, 1967a). The source document for this assessment is a Drinking Water Criteria Document for chlorobenzene (U.S. EPA, 1986). This RfD is also included on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). The HEAST (U.S. EPA, 1997) indicates the availability of the chronic RfD on IRIS, but does not list a subchronic RfD. The CARA list (U.S. EPA, 1991a, 1994a) includes a Health Effects Assessment (HEA) for chlorobenzene (U.S. EPA, 1989) that derived a subchronic RfD of 0.3 mg/kg-day and chronic RfD of 0.03 mg/kg-day based on the same 13-week dog study, as well as an Ambient Water Quality Criteria Document (U.S. EPA, 1980) and Health Assessment Document (U.S. EPA, 1985) for chlorinated benzenes, neither of which included derivation of an RfD for chlorobenzene. ATSDR (1990) derived an intermediate duration MRL of 0.4 mg/kg-day for chlorobenzene based on a NOAEL of 60 mg/kg-day and LOAEL of 125 mg/kg-day for liver effects (increases in liver weight and serum biomarkers for hepatotoxicity) in rats and mice administered chlorobenzene for 13 weeks (NTP, 1985).

No RfC is available for chlorobenzene on IRIS (U.S. EPA, 2006). The HEAST (U.S. EPA, 1997) lists a chronic RfC of 2E-2 mg/m^3 for chlorobenzene based on a subchronic study in rats (Dilley, 1977); however, this RfC was prepared using outdated methodology. A subchronic RfC for chlorobenzene is not reported in the HEAST (U.S. EPA, 1997). The source document for the RfC in the HEAST was the HEA for chlorobenzene (U.S. EPA, 1989). An RfC for chlorobenzene was not included in the Ambient Water Quality Criteria Document (U.S. EPA, 1980) or the Health Assessment Document (U.S. EPA, 1985) for chlorinated benzenes. ATSDR (1990) has not derived inhalation-based Minimal Risk Levels (MRLs) for chlorobenzene. California EPA (OEHHA, 2006) has derived a chronic inhalation REL of 1 mg/m^3 based on the occurrence of liver, kidney, and testicular lesions in a multigeneration study in rats (Nair et al., 1987). ACGIH (2006) has adopted a TLV of 10 ppm (46 mg/m^3) based on liver effects in experimental animals (Dilley, 1977; Nair et al., 1987). The OSHA (2006) PEL is 75 ppm (350 mg/m^3). NIOSH (2006) has not established a REL for chlorobenzene, but has questioned whether the OSHA PEL is adequate to protect workers from the recognized health hazards.

The cancer assessment for chlorobenzene on IRIS (U.S. EPA, 2006) includes a classification of Group D, not classifiable as to human carcinogenicity. This classification is based on no human data, inadequate animal data, and predominantly negative genetic toxicity data in bacterial, yeast, and mouse lymphoma cells. A significant positive trend was observed in the incidence of hepatocellular neoplastic nodules in male (but not female) rats administered chlorobenzene by gavage for 103 weeks; no site-specific tumors or neoplastic pathology were observed in similarly-treated mice (NTP, 1985). Quantitative estimates of carcinogenic risk from oral or inhalation exposure were not made.

The toxicity of chlorobenzene was reviewed by WHO (1991). Updated literature searches for additional toxicity data for chlorobenzene were performed for the period from 1988 to June, 2003 in the following databases: TOXLINE (supplemented with NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS, and TSCATS. The above listed documents and literature searches were used to identify relevant studies. Additional literature searches from June 2003 through October 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure. No relevant data were located regarding the toxicity of chlorobenzene to humans following oral exposure.

Inhalation Exposure. Five human inhalation studies (Rosenbaum et al., 1947; Tarkhova, 1965; Ogata et al., 1991; Girard et al., 1969; and Syrovadko and Malysheva, 1977) were located. The Tarkhova (1965) and Ogata et al. (1991) studies are acute exposure studies and Rosenbaum et al. (1947), Girard et al. (1969), and Syrovadko and Malysheva (1977) are occupational exposure studies.

In a biological monitoring study conducted by Ogata et al. (1991), 4 humans were exposed once to 60.2 ppm (277 mg/m^3) chlorobenzene for 3 hours in the morning and 4 hours in the afternoon, with a 1 hour break between the morning and afternoon exposure sessions. All of the subjects complained of a sensation of a disagreeable odor and of drowsiness, three of a heavy feeling in the head and/or headache, two of throbbing pain in the eyes, and one complained of a sore throat. The authors did not report the incidence of these effects in the control group, thus the significance of the reported symptoms is not known. A significant decrease, as compared to a non-exposed control group, in mean flicker-fusion value was observed (no further details on the control group were given). No significant alterations in pulse rate or systolic or diastolic blood pressure were found.

Tarkhova (1965) exposed 4 subjects to 0.1, 0.2, and 0.3 mg/m^3 of chlorobenzene (0.02, 0.04, and 0.07 ppm) and measured changes in electroencephalographic (EEG) patterns in response to light flashes. All subjects were exposed to all three concentrations, but the author did not indicate how much time was allowed for recovery or the order of the exposures. It appears that the experiment was repeated at least "three times during three days for each subject". The subjects were exposed to chlorobenzene for 2½ minutes in each session. The exposure period was preceded by a 3 minute control period. No effects were observed at the 0.1 mg/m^3 concentration. A response was observed in 2/4 subjects at 0.2 mg/m^3 and in 3/4 subjects at 0.3 mg/m^3.

Several occupational exposure studies suggest a neurotoxic effect in workers exposed to chlorobenzene; however, the results do not allow for a definitive conclusion because workers were exposed to other chemicals in addition to chlorobenzene. Rosenbaum et al. (1947; as reviewed by U.S. EPA, 1985 and ATSDR, 1990) examined 28 factory workers intermittently exposed to chlorobenzene for 1-2 years. Exposure concentrations were not reported. Headaches and signs of somnolence and dyspepsia were common among the workers. Tingling, numbness, and stiffness of the extremities and hyperesthesia of the hands were observed in 8 of the 28 workers and spastic contractions of the finger muscles were observed in 9 of 28 workers. Without comparative data from non-exposed workers, it is not clear that these symptoms were caused by chlorobenzene exposure. Girard et al. (1969) reported anemia and symptoms of central nervous system effects (headaches, numbness, and lethargy) and eye and respiratory tract irritation in workers exposed to chlorobenzene at unspecified concentrations. These workers, however, were also exposed to other unspecified chemicals in addition to chlorobenzene. Increased number of birth anomalies and hormonal disturbances were associated with occupational exposure of chlorobenzene and tricresol in female workers (Syrovadko and Malysheva, 1977). However, it is not possible to attribute these effects to chlorobenzene exposure because workers were exposed to tricresol in addition to chlorobenzene.

Overall, the human data suggest that chlorobenzene may affect the nervous system. However, none of the human data are adequate for use in risk assessment either because the effects cannot be definitively attributed to chlorobenzene exposure or because only acute exposures were used.

Animal Studies

Oral Exposure. Subchronic oral studies in dogs (Hazleton Laboratories, 1967a), rats (Hazleton Laboratories, 1967b; NTP, 1985; Irish, 1963; Varshavskaya, 1967), and mice (NTP, 1985), chronic studies in rats and mice (NTP, 1985), and an oral developmental toxicity study in rats (IBT, 1977) were located. These studies are described below.

Groups of 4 male and 4 female young adult beagle dogs were treated with 0, 0.025, 0.05, or 0.250 mL/kg (0, 27.5, 55.0, 275 mg/kg-day using a specific gravity of 1.1) of pure chlorobenzene via capsule 5 days/week for 13 weeks (duration-adjusted doses of 0, 19.6, 39.3 or 196.4 mg/kg-day) (Hazleton Laboratories, l967a). The dogs were observed daily for appearance and behavior, and body weight and food consumption were determined weekly. Hematology, serum chemistry, and urine analyses were performed after 1 month of treatment and again after 3 months. The dogs were sacrificed after 3 months. All dogs, including those that died during the study, were examined for gross pathology. Organ weights were determined at necropsy. Histological examination was performed for 20 organs (brain, pituitary, thyroid, lung, heart, liver, gallbladder, spleen, kidney, adrenal, stomach, pancreas, duodenum, jejuneum, ileum, colon, urinary bladder, ovaries, bone, and bone marrow) in the control and high-dose dogs, but only suspected target organs were examined in the low- and mid-dose dogs.

Four of the 8 high-dose dogs (2 males and 2 females) died or were sacrificed in moribund condition within the first 5 weeks of the study (Hazleton Laboratories, l967a). Death was preceded by loss of appetite, weight loss, inactivity, and coma. High-dose dogs that survived had reduced appetite and loss of weight over the first 5-6 weeks of the study, but appetite returned and body weight held steady over the remainder of the experiment. Terminal weight loss in these dogs ranged from 0.7 to 2.0 kg. A number of changes in blood and urine parameters were observed in dogs from the high-dose group, including low blood sugar, high circulating levels of immature leukocytes, increased urinary concentrations of acetone and bilirubin, and slight-to-marked increases in serum alkaline phosphatase, alanine aminotransferase, bilirubin, and cholesterol. Gross pathology in high-dose dogs included grey-yellow discoloration of the hepatic parenchyma, distended gallbladder, and red discoloration of the renal medulla. Increases in relative weight of the liver, kidney, adrenals, heart, and thyroid were observed among high-dose dogs, reflecting the poor physical condition of dogs in this group.

Histopathological examination of high-dose dogs revealed moderate-to-severe vacuolation, formation of fatty cysts and bile stasis in the liver, glomerular swelling and swelling and vacuolation of tubular epithelium in the kidney, variations in mucus content of the gastrointestinal mucosa, and leukocytosis and moderate-to-high cellularity in the bone marrow (Hazleton Laboratories, l967a). Incidence data for the liver and kidney lesions are reported in Table 1. Although the small group sizes in this study limit the power of statistical tests to detect changes, statistically significant increases were shown for several of the liver lesions in the high-dose group (males and females combined, Fisher exact test conducted for this review). No liver or kidney lesions were observed in control animals. Histopathological changes in the liver and kidney were the only effects observed in mid-dose dogs. These changes included slight bile duct proliferation, slight swelling and vacuolation and leukocytic infiltration in the liver, and swelling of tubular epithelium and variations in cellularity in the kidney. No effects of any type were

Table 1. Incidence of Liver and Kidney Lesions in Male and Female Dogs (Combined) Administered Chlorobenzene Daily for 13 Weeks[a] (Hazleton Laboratories, 1967a)

Organ, Lesion	Dose (mg/kg-day)			
	0	19.6	39.3	196.4
Liver, bile stasis	0/8	0/8	0/8	4/8
Liver, pigment deposition	0/8	0/8	0/8	3/8
Liver, centrilobular degeneration	0/8	0/8	0/8	8/8[b]
Liver, vacuolation	0/8	0/8	1/8	6/8[b]
Liver, cytologic changes	0/8	0/8	1/8	4/8
Liver, bile duct hyperplasia	0/8	0/8	3/8	7/8[b]
Kidney, tubular dilation	0/8	0/8	2/8	4/8
Kidney, proximal convoluted tubule swelling	0/8	0/8	0/8	2/8
Kidney, proximal convoluted tubule vacuolation	0/8	0/8	1/8	4/8
Kidney, tubule epithelial degeneration	0/8	0/8	1/8	4/8
Kidney, terminal proximal tubule vacuolation	0/8	1/8	0/8	3/8
Kidney, epithelial pigment deposition	0/8	0/8	0/8	3/8

[a] Data reported as number of animals observed with the lesion/total number of animals in the dose group
[b] Incidence significantly greater than controls using the Fisher exact test ($p<0.05$) performed for this review

observed in low-dose dogs. This study, therefore, identified a LOAEL of 39.3 mg/kg-day for liver and kidney effects (histopathological changes) and a NOAEL of 19.6 mg/kg-day.

In a companion study to Hazleton Laboratories (l967a), Charles River CD rats (18/sex/group) were given 12.5, 50, 100, or 250 mg/kg-day of pure chlorobenzene by gavage in corn oil, daily for 93-99 days (Hazleton Laboratories, 1967b; Knapp et al., 1971). An additional group of 18 males and 18 females served as an untreated control group. The rats were observed daily for appearance and behavior. Rats in the test groups were weighed daily, while those in the control group were weighed weekly. Food consumption was determined weekly. Hematology, clinical chemistry and urine analyses were performed at 30 and 90 days using 5 males and 5 females from each group. Sacrifice was performed after 93-99 days of treatment. All animals, even those that died during the study, received a gross necropsy. Organ weights were determined at necropsy. Histopathological examination (on 5 males and 5 females from each group) included 17 organs (brain, pituitary, thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, intestines, urinary bladder, gonads, femur, and bone marrow) in the high-dose and control groups, but only the thyroid, heart, liver, kidney and adrenals were examined from the other groups. Although a few deaths occurred during the course of the study, there was no clear relationship between treatment and mortality. There was a statistically significant decrease in body weight gain among high-dose males (terminal body weight reduced approximately 7%), but growth was not affected in other groups. Food consumption did not differ from controls. The only clinical sign clearly related to treatment was salivation following dosing throughout the first week of the study. Salivation generally occurred in about half of the rats exposed to 50 mg/kg-day, a majority of those exposed to 100 mg/kg-day and all of those exposed to 250 mg/kg-day. Hematology, clinical chemistry and urinalysis results were unremarkable. The only gross pathological observation of interest was a high incidence of mottled and discolored livers in rats exposed to 50, 100, or 250 mg/kg-day, that did not, however, increase in incidence or intensity as dose increased from 50 to 250 mg/kg-day. Absolute and relative liver weights were significantly increased in females exposed to 100 or 250 mg/kg-day and males exposed to 250 mg/kg-day. Absolute and relative kidney weights were also significantly increased at these doses. Histopathological examination failed to detect any compound-related effects in rats of any dose group. NOAEL and LOAEL values of 50 and 100 mg/kg-day, respectively, may be derived from this study based on weight increases in the liver and kidney. Although the organ weight increases were not accompanied by histopathological changes or other clear indicators of toxicity, the results of the companion study on dogs (Hazleton Laboratories, 1967a) showed that these organs are targets of chlorobenzene toxicity.

Supporting data also come from subchronic and chronic studies in rats and mice conducted by NTP (1985). In the subchronic studies, groups of 10 F344/N rats and 10 B6C3F1 mice of each sex were given chlorobenzene at 0, 60, 125, 250, 500, or 750 mg/kg-day, 5 days/week for 13 weeks by gavage in corn oil. Duration-adjusted doses were 0, 43, 89, 179, 357 or 536 mg/kg-day, respectively. Clinical signs of toxicity were noted daily and body weights were determined weekly. Urine samples for analysis were obtained during the 13th week of treatment. Blood samples for hematology and clinical chemistry analyses were collected prior to sacrifice. The major organs were weighed at necropsy. Comprehensive histopathological examinations were given to rats from the two highest dose groups and mice from the three highest dose groups, as well as controls. Only suspected target organs were examined

microscopically in the other groups. Chlorobenzene produced death in rats exposed to 500 mg/kg-day (4/10 males and 3/10 females) and 750 mg/kg-day (9/10 males and 8/10 females). Body weight gain was reduced about 20% in both males and females from these groups. The most frequent histopathological lesions in these groups were moderate centrilobular hepatocellular necrosis, mild-to-moderate nephrosis (characterized by degeneration and necrosis of the proximal tubule) and minimal-to-moderate myeloid depletion of the bone marrow. Other lesions observed were hepatic degeneration and lymphoid depletions of the thymus and spleen. Further effects observed in rats from these dose groups included decreased white blood cell count, increased reticulocytes, increased serum alkaline phosphatase and gamma glutamyl transpeptidase, increased urinary output, increased urinary excretion of uroporphyrin and coproporphyrin, increased absolute and/or relative liver and kidney weights and decreased absolute and relative spleen weight. Effects in rats exposed to 250 mg/kg-day included reduced body weight gain (>20%, males only), increased absolute and relative liver weight, decreased absolute and relative spleen weight and a few observations of minimal hepatic necrosis and nephropathy. The only effects at lower doses were increased absolute and relative liver weight in females exposed to 125 mg/kg-day and decreased absolute and relative spleen weight in males exposed to 60 or 125 mg/kg-day. Taken together, the liver was identified as the most sensitive target organ. Increased liver weight was observed at $\geq$125 mg/kg-day, and hepatic necrosis occurred at $\geq$250 mg/kg-day. Although spleen weights were decreased at all doses, microscopic lesions (lymphoid depletion) were only observed at the high dose (750 mg/kg-day). Therefore, these results suggest a NOAEL and LOAEL in rats of 60 and 125 mg/kg-day, respectively (43 and 89 mg/kg-day, respectively, when adjusted for a 5 day/week dosing schedule).

Results in mice were similar in pattern to those in rats, although mice appeared to be more sensitive to chlorobenzene toxicity, as indicated by an increase in mortality in this species at 250 mg/kg-day (5/9 males and 4/10 females) and above (37/40 mice) (NTP, 1985). Body weight gain was reduced 50-80% in these groups. Histopathological lesions were generally limited in occurrence to these same dose groups; lesions included severe hepatic necrosis, moderate renal tubular necrosis, myeloid depletion of the spleen and bone marrow, lymphoid depletion of the spleen and thymus, and necrosis of the thymus. Absolute and relative liver weights were significantly increased in surviving males and females from these groups. Other changes in these dose groups were increased urinary output and increased urinary excretion of coproporphyrins. The only effect in mice exposed to 125 mg/kg was significantly increased absolute and relative liver weight in males. Based on liver toxicity in mice, NOAEL and LOAEL values of 60 and 125 mg/kg-day (duration adjusted doses of 43 and 89 mg/kg-day) can be derived.

In the chronic studies, groups of 50 rats of each sex and 50 female mice were administered chlorobenzene by gavage in corn oil at 0, 60 or 120 mg/kg-day 5 days/week for 103 weeks (NTP, 1985). Duration-adjusted doses were 0, 43, and 86 mg/kg-day, respectively. Groups of 50 male mice were similarly treated with 0, 30 or 60 mg/kg-day (duration adjusted doses of 0, 21, 43 mg/kg-day, respectively). Survival was significantly reduced in male rats in the 120 mg/kg group, but not in lower dose male rats or female rats. Body weight gain was not affected in rats of any group. From the original microscopic examination, there appeared to be a slightly increased incidence of hepatic necrosis in treated rats of both sexes (males at 60 mg/kg and females at 120 mg/kg), but a second independent review did not support these findings.

Other than effects on immune system at substantially high doses, no other chemical-related non-carcinogenic effects were identified in the rats. In mice, survival was marginally reduced in males at 30 and 60 mg/kg; however, survival trend did not follow a dose-response relationship and no effect was noted in females. Body weight gain was similar in treated and control mice and no treatment- related non-neoplastic lesions were identified.

Irish (1963) briefly reported the results of an unpublished Dow Chemical study, in which rats were treated orally with chlorobenzene 5 days per week for approximately 6 months. Doses of 144 and 288 mg/kg-day (duration adjusted doses of 103 and 206 mg/kg-day) produced significant increases in liver and kidney weight and slight liver pathology. No effects were detected in rats treated with 14.4 mg/kg-day (duration adjusted dose of 10.3 mg/kg-day). Further details regarding this study were not provided.

In contrast to the results of the studies described above, toxicity was reported at much lower doses by Varshavskaya (1967). Groups of 7 male albino rats weighing 180-200 g were treated with 0, 0.001, 0.01 or 0.1 mg/kg-day of chlorobenzene in sunflower oil by stomach tube for 9 months. Effects reported at 0.1 mg/kg-day included inhibition of higher nervous system activity (i.e., prolonged formation and accelerated loss of conditioned reflexes), a statistically significant inhibition of erythropoiesis (i.e., decreased red blood cell count and hemoglobin), increased serum alkaline phosphatase and aminotransferase levels, and immune system effects (increased leukocytes and gamma globulin). Many of these endpoints were also marginally affected by exposure to 0.01 mg/kg-day. No effects were reported at 0.001 mg/kg-day. Although some of the effects reported in this study are consistent with those observed in other studies, the effective doses are much lower. Varshavskaya (1967) also reports effects for o-dichlorobenzene that are over 3 orders of magnitude lower than other published values. Therefore, U.S. EPA (1980, 1985) considered the results of this study to be questionable.

Chlorobenzene was the subject of a developmental toxicity study in rats (IBT, 1977). Pregnant Charles River albino rats (20-22 per dose) were administered chlorobenzene at 100 or 300 mg/kg-day on gestation days 6-15 via oral gavage. Maternal body weight, mortality, and clinical signs of toxicity were recorded at regular intervals throughout exposure. All dams were sacrificed on gestation day 20 and were administered via Caesarian section. Implantation sites and the number of corpora lutea were determined, and the number of viable fetuses was recorded. All fetuses were removed from the uterus, weighed, and examined for external malformations. Two-thirds of the fetuses were examined for skeletal effects; the remaining fetuses were evaluated for internal development. No treatment-related effects were noted at any dose; however, the study did not test up to maternally toxic doses. The results of this study do not rule out developmental effects at high doses, but indicate that developmental toxicity is not likely a sensitive toxicological endpoint for chlorobenzene toxicity.

Inhalation Exposure. Several studies have examined the subchronic toxicity of inhaled chlorobenzene in animals (IBT, 1979; Roloff, 1980; Dilley, 1977; Irish, 1963; Zub, 1978). John et al. (1984) and Nair et al. (1987) have examined the developmental and reproductive toxicity, respectively, of inhaled chlorobenzene.

In a study conducted by IBT (1979), groups of male and female rats (15/sex/group) and beagle dogs (4/sex/group) were exposed to 0, 0.76, 1.47, or 2 mg/L (0, 760, 1470, or 2000 mg/m^3) of chlorobenzene 6 hours/day, 5 days/week for 90 days (62 exposure days). Controls were exposed to "clean air." All animals were observed for mortality and clinical signs of toxicity daily throughout the exposure period, and body weights were recorded weekly. Blood was taken from all surviving dogs at Day 28 (blood was taken from several dogs earlier than Day 28 because they were expected to be sacrificed moribund prior to the bleed) and from 5 control and high-concentration rats per sex at Days 39 and 91. Hematology, clinical chemistry, and urinalysis examinations were conducted at each bleed. At scheduled sacrifice, all animals were subjected to a gross pathology evaluation, the adrenal glands (dogs only), brain (cerebrum, cerebellum, and pons), lungs, pancreas, pituitary gland (dogs only), spleen, and thyroid gland (dogs only) were weighed (absolute weights and organ weight relative to the brain and terminal body weight were determined), and 29-32 tissues were microscopically examined from the control and high-concentration groups. Tissues from low- and mid-concentration animals were examined only if "significant pathologic findings" were observed at the high concentration.

No effects on rats were observed for any of the parameters evaluated (IBT, 1979). In dogs, however, a number of potential treatment-related effects were observed. An apparent concentration-related increase in mortality was observed. Mortality rates in dogs exposed at 0, 760, 1470, and 2000 mg/m^3, respectively, were 0/4, 0/4, 1/4, and 2/4 in males and 0/4, 0/4, 1/4, and 3/4 in females. Hypoactivity was observed in 0/4, 1/4, and 4/4 dogs at the low-, mid-, and high-concentrations, respectively, in both males and females (control incidences were not reported). Conjunctivitis occurred at the same incidence rates in both males and females. Also, one high-concentration female dog was observed with glazed eyes. There were no clear effects on body weight, although final mean body weights of high-concentration dogs were less than controls. No chlorobenzene-related alterations in hematological, serum clinical chemistry, or urinalysis parameters were observed. A number of statistically significant changes in absolute and relative organ weights were found; however, only pancreas weights of female dogs appeared to show a concentration-response relationship (although the lack of several organ weights from the low- and mid-concentration groups precludes a full evaluation of potential treatment-related effects). The toxicological relevance of the change in pancreas weight, however, is not clear because no microscopic lesions were observed in the pancreas.

Icterus (characterized by yellow discoloration of the aorta) and enlarged hardened livers were observed in dogs that were killed *in extremis* (IBT, 1979). Microscopic lesions were observed in the liver, kidney, testes, and bone marrow in treated dogs. At 2000 mg/m^3, slight to moderate vacuolation of the liver (2/4 males, 3/4 females), aplastic bone marrow (2/4 males, 3/4 females), epithelial cytoplasmic vacuolation in the kidneys (1/4 males, 3/4 females), and atrophy of the seminiferous epithelium in the testes (2/4 males) were observed. At 1470 mg/m^3, vacuolation of the liver (1/4 males) and juvenile testes (1/4 males) were observed. These lesions were not seen in controls. No tissues from low-concentration males or females were microscopically examined. This study was not peer-reviewed, and statements from the researchers highlighted that only a limited quality assurance review was given to this report. Therefore, reliable NOAEL or LOAEL values cannot be derived from this study. However, the study does provide suggestive evidence that the liver, kidneys, bone marrow, and testes may be target organs for chlorobenzene in dogs.

As a follow-up study, Roloff (1980) exposed beagle dogs (6 per sex and concentration) to chlorobenzene (96.5% pure) 6 hours/day, 5 days/week for 6 months at 0, 0.79, 1.59, or 2.06 mg/L (0, 790, 1590, or 2060 mg/m^3). Clinical signs of toxicity were recorded at regular intervals during the 6-hour exposure periods, detailed physical examinations were conducted weekly, and body weight was recorded weekly. After six months of exposure, animals were sacrificed, the adrenals, brain, heart, kidney, liver, pituitary, and testes were weighed, and 24 tissues were microscopically examined. A number of hematology, clinical chemistry, and urinalysis parameters were determined twice prior to study initiation, twice during the first four weeks of exposure, monthly thereafter, and at terminal sacrifice.

Body weight, food consumption, and general health of the dogs were unaffected by chlorobenzene exposure (Roloff, 1980). A concentration-related, statistically significant increase in the number of dogs observed to vomit ($p \leq 0.01$) or pass abnormal stools ($p \leq 0.01$) was reported, suggesting gastrointestinal irritation at all concentrations. However, histopathology did not reveal any treatment-related lesions of the GI tract, and other studies have not observed gastrointestinal effects. Therefore, the toxicological significance of this observation is not clear. A statistically significant ($p \leq 0.05$) increase in liver-to-body weight ratio was observed in mid- and high-concentration females and a significant ($p \leq 0.05$) decrease in absolute adrenal weight was observed in the mid- and high-concentration males. Kidney weight was not affected by treatment. In the absence of microscopic lesions in these tissues, the biological significance of the organ weight changes is not clear. Although chlorobenzene exposure has been shown to affect the liver in other studies, only relative weights in females were significantly increased compared with controls and no microscopic lesions were observed in the liver in this study. Also, relative liver weights in females did not show a clear concentration-related increase. Relative liver weights at 0, 790, 1590, and 2000 mg/m^3, respectively, were 2.4%, 3.2%, 3.1%, and 3.1%. Therefore, it does not appear that the increase in relative liver weight in female dogs was related to treatment. Statistically significant changes in various clinical chemistry parameters were observed; however, these changes appeared to be random and not related to chlorobenzene exposure. A clear LOAEL was not observed in this study.

Taken together, these 90 day and 6-month studies in dogs resulted in contradictory results and, therefore, do not allow for reliable NOAEL or LOAEL derivations. In one study, (IBT, 1979), an apparent concentration-related increase in mortality and effects on the kidney, liver, and testes were observed. In a follow-up study (Roloff, 1980), however, no effects were observed in dogs at comparable concentrations. Therefore, a clear NOAEL or LOAEL in dogs was not established.

In a study reported by Irish (1963), groups of rats, rabbits, and guinea pigs were exposed to 0, 200, 475, or 1000 ppm (0, 920, 2189, or 4604 mg/m^3) of chlorobenzene for 7 hours/day, 5 days/week for 44 days. In the guinea pigs exposed at 4604 mg/m^3, increased mortality was observed. Unspecified histological alterations were observed in the liver, kidney, and lungs in exposed animals at 4604 mg/m^3 (the study report did not specify which effects were associated with each species tested). At 2189 mg/m^3, slight histological alterations were also observed in the liver. No effects were observed at 920 mg/m^3. Additional details were not available.

Zub (1978) exposed male and female white Swiss mice (5 per sex and concentration) to chlorobenzene vapors at 100 mg/m^3 daily (7 hours per day) for 3 months or 2500 mg/m^3 daily for 3 weeks. Additional experimental design parameters were not reported. Five of 10 mice exposed at 2500 mg/m^3 died. Loss of appetite, general emaciation, marked somnolence, decreased body weight, fatty degeneration and atrophy in the liver were also observed at 2500 mg/m^3. Slight leukopenia and lymphocytosis were the only haematological effects in mice exposed to 100 mg/m^3 for 3 months. Although these data support the conclusion that chlorobenzene may affect the liver, the data are limited because sufficient detail on experimental methods and results were not reported in the published article to permit critical evaluation of the study.

Dilley (1977) exposed groups of 32 male Sprague-Dawley rats and 32 male rabbits (strain not specified) to 0, 73, or 248 ppm (0, 336, or 1142 mg/m^3, respectively) of chlorobenzene for 7 hours/day, 5 days/week for 24 weeks. Groups of 10 rats and 10 rabbits were killed after 5, 11, or 24 weeks of exposure. Animals were weighed weekly for 5 weeks, every 2 weeks for the next 4 weeks and monthly thereafter. All animals were observed daily for clinical signs of toxicity. The brain, heart, lungs, liver, spleen, kidneys, and gonads were weighed. These tissues and the adrenal glands, bone marrow, eye, skin, and abnormal tissues were microscopically examined. A number of hematology and clinical chemistry parameters were evaluated.

In rats, no deaths, unusual clinical observations or changes in body weight gain were observed (Dilley, 1977). The kidney and liver weights generally increased with increasing concentration (Table 2). Significant increases in absolute and relative liver weights were observed in male rats exposed to 248 ppm for 24 weeks (Table 2) compared with controls. Relative kidney weights were also significantly greater than controls after 24 weeks.

Hematology evaluations found decreased hematocrit and mean corpuscular volume, and increased mean corpuscular hemoglobin concentration, in rats exposed to chlorobenzene at $\geq$73 ppm after 11 weeks of exposure, consistent with microcytic anemia; however, similar effects were not observed at 24 weeks (Dilley, 1977). Therefore, the biological significance of this observation is not clear. The only consistent and significant change in the rat clinical chemistry profile was reduced serum aspartate aminotransferase (AST) activity in the high-dose group at all three sacrifice times. The toxicological significance of this observation is not clear. Histopathology revealed no consistent concentration-related increase in the incidences of any lesions. Chronic respiratory disease was observed in 8-10 rats in all treatment and control groups. It is not known if the chronic respiratory disease made the animals unusually sensitive to the toxicity of chlorobenzene or masked some aspects of chlorobenzene toxicity. Therefore, a NOAEL and LOAEL suitable for RfC derivation cannot be identified from this study. However, the organ weight data provide supportive evidence that the liver and kidneys are possible targets of chlorobenzene toxicity.

Table 2. Selected Organ Weights in Male Rats Exposed to Chlorobenzene via Inhalation for 24 Weeks (Dilley, 1977)

Organ	Absolute or Relative Weight	0 ppm	73 ppm	248 ppm
Liver	Absolute (g)	16±0.8[a]	18±0.9	21±2.0[b]
	Relative, body	34±0.6	38±1.0	44±1.9[c]
	Relative, brain	7.3±0.4	8.0±0.4	9.7±0.8
Kidneys	Absolute (g)	3.5±0.2	3.7±0.1	4.1±0.2
	Relative, body	7.5±0.2	7.9±0.2	8.5±0.2[b]
	Relative, brain	1.6±0.08	1.6±0.06	1.8±0.08

[a] mean ± standard deviation
[b] statistically significant ($p \leq 0.05$)
[c] statistically significant ($p \leq 0.01$)

In rabbits, no treatment-related deaths, unusual clinical observations, or changes in body weight gain were observed (Dilley, 1977). Overall, there were no consistent concentration-related changes in hematology, clinical chemistry, or gross or microscopic lesions. Encephalitozoonosis (caused by *Escherichia cuniculi* infection) and respiratory illness associated with atelectasis and emphysema; lymphocytic foci near bronchi and bronchioles, focal edema and congestion were observed in a number of treated and control animals that may have affected the rabbits' sensitivity to chlorobenzene-induced toxicity. Therefore, these data are not suitable for RfC derivation.

Nair et al. (1987) conducted a two-generation reproductive study in rats. In this study, groups of 30 male and 30 female CD Sprague-Dawley rats were exposed to chlorobenzene (>99.9% pure) in a dynamic air chamber at target concentrations of 0, 50, 150, or 450 ppm (0, 230, 691, or 2072 mg/m^3) for 6 hours/day, 7 days/week for 10 weeks before mating, and during mating, gestation, and lactation. The male and female F_0 rats were sacrificed after the lactation period. Groups of 30 male and 30 female F_1 rats were exposed to the same concentrations of chlorobenzene (beginning 1 week post-weaning) for 11 weeks before mating and during mating, gestation, and lactation. The F_1 rats were also sacrificed after the lactation period. The F_2 pups were sacrificed after weaning. Mortality and clinical signs of toxicity were recorded twice each day, detailed physical examinations were conducted weekly, body weights were recorded weekly except that female body weights were also recorded at additional regular intervals throughout gestation and lactation, and food consumption was recorded weekly during the growth period. Complete gross postmortem examinations were conducted on all sacrificed animals. Liver and brain weights of F_0 and F_1 adults were recorded. Liver, kidneys, pituitary gland, and reproductive organs (males: testes, epididymides, seminal vesicle, and prostate; females: vagina, uterus, and ovaries) were examined microscopically for all F_0 and F_1 adult animals in the control and high-concentration groups. Liver, kidneys, and testes of male rats in the low- and mid-

concentration groups were microscopically examined. Hematology or clinical chemistry parameters were not evaluated.

No deaths were observed in the F_0 or F_1 groups, and no significant alterations in body weight gain were observed (Nair et al., 1987). No apparent alterations in the mating, pregnancy (number of pregnant females/number mated), fertility, pup viability, pup survival, or litter survival indices were observed in the F_0 or F_1 rats. Absolute and relative liver weights were clearly and significantly increased in F_0 and F_1 male rats exposed to $\geq$150 ppm and F_0 and F_1 female rats exposed to $\geq$450 ppm (Table 3). Much smaller, but still statistically significant, increases in relative liver weight at lower doses were consistent with the observed trend, but do not themselves indicate a toxicologically significant effect at the lower doses. Histopathology examinations identified the liver, kidneys, and testes as target organs for chlorobenzene in male rats. Table 4 shows incidence data reported by the investigators and the results of statistical tests conducted for this review (statistical tests of the incidence data were not performed by the original investigators). In the liver, the incidence of centrilobular hepatocellular hypertrophy was significantly increased in the 150 and 450 ppm F_0 males in a dose-related manner, and marginally increased in the 450 ppm F_1 males. In the kidneys, significant increases in the incidences of tubular dilation, chronic interstitial nephritis, and foci of regenerative epithelium were observed at 150 and 450 ppm in the F_0 males, but primarily at 450 ppm in the F_1 males. The incidence of small and flaccid testes was significantly increased in the F_1 males at 450 ppm, and was also observed in both F_0 and F_1 males at 150 ppm. Degeneration of the testicular germinal epithelium was seen in F_0 and F_1 males at 150 and 450 ppm, and appears to have been treatment-related. Although incidence levels were low at 150 ppm and just approached statistical significance at 450 ppm, the lesion was graded as moderate or severe in 1 F_0 and 2 F_1 males at 150 ppm and in 3 F_0 and 5 F_1 males at 450 ppm. The two observations of this lesion in controls were both graded as minimal. No concentration-related microscopic lesions were observed in female rats.

The kidney lesions observed in this study included tubular dilation, chronic interstitial nephritis, and foci of regenerative epithelium (Nair et al, 1987). Because the lesions observed in this study only occurred in male rats and are consistent with those typical of alpha-$_{2u}$-globulin accumulation (U.S. EPA, 1991b), and because other chlorobenzene derivatives have been shown to cause alpha-$_{2u}$-globulin accumulation (WHO, 1991), it is possible that the kidney effects observed in this study may not be relevant to human health risk assessment. However, there is insufficient evidence to attribute the kidney effects observed in this study to alpha-$_{2u}$-globulin accumulation. The presence of alpha-$_{2u}$-globulin was not tested for in the Nair et al. (1987) study, and other studies have demonstrated the occurrence of kidney effects in animals other than male rats. Hazleton Laboratories (1967a) reported kidney effects in orally treated dogs (including tubule dilation, vacuolation, and leukocytic infiltration), and NTP (1985) reported kidney effects in male and female mice (tubular necrosis) and male and female rats (degeneration and necrosis of the proximal tubule) orally administered chlorobenzene in subchronic studies. It is possible that the absence of kidney lesions in female rats in the Nair et al. (1987) study was due to generally lower sensitivity of the females to chlorobenzene toxicity, as liver lesions were also observed only in males in this study. Therefore, there is insufficient evidence to attribute the kidney lesions observed in this study to alpha-$_{2u}$-globulin accumulation, and the kidney

Table 3. Mean Absolute and Relative Liver Weights of F_0 and F_1 Rats Exposed to Chlorobenzene Vapor (Nair et al., 1987)

Concentration (ppm)	Males		Females	
	Absolute Liver Weight (g)	Relative Liver Weight (g)	Absolute Liver Weight (g)	Relative Liver Weight (g)
F_0 Animals				
0	19.3±2.2[a]	3.6±0.35	11.5±1.3	3.8±0.30
50	19.0±3.1	3.6±0.34	12.0±1.3	3.9±0.23
150	21.5±2.3[c]	4.1±0.30[c]	12.1±1.1	4.0±0.21[b]
450	21.9±3.8[c]	4.1±0.61[c]	13.3±1.5[c]	4.4±0.33[c]
F_1 Animals				
0	18.3±2.2	3.5±0.32	12.4±2.3	4.2±0.60
50	19.5±2.6	3.7±0.36[b]	12.7±1.6	4.2±0.35
150	21.7±3.5[c]	4.2±0.46[c]	13.1±1.6	4.4±0.41
450	23.4±4.1[c]	4.4±0.40[c]	14.0±2.0[c]	4.6±0.37[c]

[a] mean ± standard deviation
[b] statistically significant ($p \leq 0.05$)
[c] statistically significant ($p \leq 0.01$)

Table 4. Incidences of Liver, Kidney, and Testicular Lesions Observed in Adult Male Rats Exposed to Chlorobenzene via Inhalation in a 2-Generation Reproductive Toxicity Study (Nair et al., 1987)[a]

Organ, Lesion	Generation	Concentration (ppm)			
		0	*50*	*150*	*450*
Liver, hepatocellular hypertrophy	F_0	0/30[b]	0/30	5/30[c]	14/30[d]
	F_1	2/30	0/30	3/30	7/30
Kidney, tubular dilation/eosinophilic material (unilateral or bilateral)	F_0	0/30	4/30	6/30[c]	18/30[d]
	F_1	8/30	7/30	14/30	22/30[d]
Kidney, chronic interstitial nephritis (unilateral or bilateral)	F_0	1/30	2/30	7/30[c]	10/30[d]
	F_1	1/30	3/30	7/30[c]	11/30[d]
Kidney, foci of regenerative epithelium (unilateral or bilateral)	F_0	0/30	1/30	5/30[c]	8/30[d]
	F_1	1/30	0/30	5/30	11/30[d]
Testes, small and flaccid	F_0	0/30	0/30	1/30	3/30
	F_1	0/30	0/30	1/30	5/30[c]
Testes, degeneration of germinal epithelium	F_0	1/30	0/30	2/30	6/30
	F_1	1/30	0/30	3/30	6/30

[a] statistical analysis (Fisher Exact test) performed for this review and not by original investigators
[b] number of animals with lesion/total number of animals exposed
[c] statistically significant ($p \leq 0.05$)
[d] statistically significant ($p \leq 0.01$)

effects are considered relevant to human health risk assessment until conclusive evidence is obtained indicating otherwise.

Nair et al. (1987) demonstrated dose-related effects on the liver, kidney, and testes. Male rats were more sensitive than females. In all three organs, there was some evidence for an effect at 150 ppm, and more clear evidence at 450 ppm. In the liver, significant increases in liver weight and the incidence of hepatocellular hypertrophy were seen at 150 and 450 ppm. In the kidneys, the incidences of tubular dilation, chronic interstitial nephritis, and foci of regenerative epithelium were increased at both 150 and 450 ppm. The kidney effects are considered relevant to human health risk assessment, as previously discussed. In the testes, degeneration of the germinal epithelium was not statistically increased in incidence even in the 450 ppm group, but appeared to be related to treatment in both the 150 and 450 ppm groups based on severity of the lesions observed. Although the testes appeared to be a target for chlorobenzene, reproductive

performance was not affected at any exposure level. Based on these endpoints, this study identified a LOAEL of 150 ppm (691 mg/m^3) and NOAEL of 50 ppm (230 mg/m^3).

Chlorobenzene was the subject of several developmental toxicity studies. John et al. (1984) exposed groups of 32-33 pregnant Fischer 344 rats to 0, 75, 210, or 590 ppm (0, 345, 967, or 2716 mg/m^3) of chlorobenzene 6 hours/day on gestation days (GDs) 6-15. The dams were sacrificed on GD 21. At necropsy, the uterine horns were examined for (1) number and position of fetuses; (2) number of live and dead fetuses; (3) number and position of resorption sites; (4) number of corpora lutea; (5) the sex, body weight, and crown-rump length of each fetus; and (6) gross external abnormalities. One half of each litter was examined under a dissecting microscope for soft tissue alterations, and the heads of these animals were also examined by sectioning. All fetuses were examined for skeletal alterations.

No maternal deaths or changes in general appearance or behavior were observed in the chlorobenzene-exposed rats (John et al., 1984). In dams exposed to 590 ppm, significant decreases in body weight gain were observed on GDs 6-8 (Table 5); however, weight gains over subsequent intervals and total weight gains over GDs 6-20 were not significantly affected. Significant increases in absolute and relative liver weights were observed at 590 ppm (Table 5). Mean litter size and incidence of resorptions were not affected by chlorobenzene exposure, and no alterations in the incidence of malformations were observed in the rat fetuses. The incidences of some minor skeletal variations were altered in some groups, but no consistent concentration-related changes were observed. Therefore, chlorobenzene was not considered a developmental toxicant in this study. A maternal NOAEL and LOAEL of 210 ppm and 590 ppm, respectively, was identified from this study based on increased maternal liver weight and decreased body weight. The developmental NOAEL was 590 ppm, the highest concentration tested.

Table 5. Body and Liver Weights of Female Rats Exposed to Chlorobenzene (John et al., 1984)

Concentration (ppm)	Body weight gain GD 6-8[a] (g)	Liver weight (absolute) (g)	Liver weight (relative)
0	3 ± 2[b]	9.8 ±1.1	3.8 ± 0.28
75	4 ± 3	10.0 ± 0.81	3.9 ± 0.28
210	2 ± 3	10.1 ± 0.54	3.9 ± 0.21
590	-2 ± 5[c]	11.0 ± 0.83[c]	4.3 ± 0.42[c]

[a] body weight gains at other time periods were comparable to controls and are not reported
[b] mean ± standard deviation[c] statistically significant ($p \leq 0.05$)

John et al. (1984) conducted two developmental toxicity studies in rabbits. In the first study, groups of 30 pregnant New Zealand white rabbits were exposed to 0, 75, 210, or 590 ppm (0, 345, 967, or 2716 mg/m^3) of chlorobenzene for 6 hours/day on GDs 6-18 and sacrificed on GD 29. Other details of the protocol were the same as described for rats (John et al. 1984). No

effect on body weight or weight gain was observed in the does. Absolute and relative liver weights in the does were reported to be significantly increased at 210 and 590 ppm, but the data were not shown. No effects on reproductive or fetal parameters were found. There was a statistically significant ($p \leq 0.05$) increase in the incidence of fetuses with extra rib at 590 ppm. The number of litters affected, however, was comparable to controls (Table 6). There were also several observations of historically rare malformations (head/facial anomalies, heart defects, spina bifida, acephaly) in treated rabbits that were not seen in controls (Table 6). Because it was not clear that any of these effects were directly related to chlorobenzene treatment, a second experiment was conducted in rabbits at 0, 10, 30, 75, and 590 ppm.

Table 6. Fetal Alterations in Chlorobenzene Exposed Rabbits - Experiment 1 (John et al., 1984)

Concentration (ppm)	Extra Rib	Head/Facial Anomalies	Heart Anomalies	Spina Bifida	Acephaly
0	79 (24)[a]	0	0	0	0
75	68 (19)	1 (1)	0	0	0
210	92 (33)	0	1 (1)	1 (1)	0
590	113[b] (26)	1 (1)	2 (2)	1 (1)	1 (1)

[a] number of fetuses affected (number of litters affected in parentheses)
[b] statistically significant ($p \leq 0.05$)

In the second study (John et al., 1984), groups of 30-32 pregnant New Zealand White rabbits were exposed to 0, 10, 30, 75, or 590 ppm (0, 46, 138, 345, or 2716 mg/m^3) of chlorobenzene 6 hours/day on GDs 6-18. An increase in maternal liver weight was observed in the 590 ppm group. No significant alterations in the number of litters, number of fetuses per litter, or the number of implantations resorbed were observed; however, there was a significant increase in the number of litters with resorptions at 590 ppm. This observation, however, was not considered to be related to chlorobenzene exposure by the researchers because the incidence was within the range of historical controls (details on the historical controls were not reported) and because this effect was not observed in the first rabbit study. The incidence of malformations was not altered in the chlorobenzene-exposed groups. The malformations observed in the first rabbit study were either not observed at all in the second study or were seen at comparable incidence in the control group. An increased incidence of fetuses with extra ribs was found at 10 ppm, but the number of affected litters was similar to controls. No increases in extra ribs were seen at $\geq$30 ppm. Overall, no consistent developmental effects were observed in the two studies conducted in rabbits. The NOAEL for developmental toxicity was the high concentration of 590 ppm. Increased liver weights were observed at $\geq$210 ppm in maternal animals in the first study; the second study did not test any concentration between 75 and 590 ppm. Therefore, the maternal NOAEL for these studies was 75 ppm (345 mg/m^3) and the maternal LOAEL was 210 ppm (967 mg/m^3), based on increased liver weights.

Tarkhova (1965) exposed groups of 15 male white rats (strain not specified) to 0, 0.1, or 1.0 mg/m^3 (0, 0.02, 0.2 ppm) for an "uninterrupted" 60-day period. No alterations in body weight or appearance were observed. In the 1.0 mg/m^3 group, the conduction speeds of nerve impulses to sets of flexor and extensor muscles had changed on day 39. The ratios of chronaxias of the flexor and extensor muscles in the 1 mg/m^3 exposed animals were measured every 9-10 days as the experiment progressed. Ninety-nine percent (99%) reliability of changes by comparison to the control were observed starting day 39. A significant increase in blood cholinesterase and changes in the ratio of albumin:α-globulin ratio (direction of the change can not be determined) was also observed in the 1.0 mg/m^3 group. The rise in blood cholinestrase activity was observed in the 1 mg/m^3 exposed groups of animals on the 36th day of the treatment.

Aranyi et al. (1986) tested the immunotoxicity of a number of potentially hazardous air contaminants, including chlorobenzene. Female CD_1 mice (135/group) 4-5 weeks old were exposed to either 0 or 75 ppm (0 or 345 mg/m^3) of chlorobenzene for 3 hours/day for 5 days. The mice were exposed simultaneously to an aerosol of viable *Streptococcus zooepidemicus*, and deaths over a 14-day observation period were recorded. Pulmonary bactericidal activity of *in vivo* alveolar macrophages was also monitored in animals (23/group) simultaneously exposed to ^{35}S-*Klebsiella pneumonia* and either chlorobenzene or air. The ratio of viable bacterial counts to radiolabeled bacteria was used to determine bactericidal activity. Exposure to chlorobenzene resulted in no significant increase in mortality in female CD_1 mice due to *S. zooepidemicus* challenge after 5 days of simultaneous exposure for 3 hours/day, in comparison with filtered-air controls. There was also no evidence of any adverse effect on the bactericidal activity of alveolar macrophages due to chlorobenzene exposure. The data indicate that immunotoxicity is not likely a sensitive toxicological endpoint for chlorobenzene.

DERIVATION OF A PROVISIONAL SUBCHRONIC RfD FOR CHLOROBENZENE

No relevant data were located regarding the subchronic or chronic toxicity of chlorobenzene to humans following oral exposure. Subchronic studies in dogs (Hazleton Laboratories, 1967a), rats (Hazleton Laboratories, 1967a; NTP, 1985; Irish, 1963; Varshavskaya, 1967), and mice (NTP, 1985) and chronic studies in rats and mice (NTP, 1985) were located. Overall, the data indicate that the liver and kidneys are the most sensitive target organs of orally administered chlorobenzene in experimental animals, and that the dog is the most sensitive species evaluated to chlorobenzene toxicity. In dogs, increased incidence of liver and kidney pathology was reported by Hazleton Laboratories (1967a) at ≥39.3 mg/kg-day. Effects on the bone marrow and GI tract were observed at higher chlorobenzene doses. In rodents, increased liver and kidney weights and liver pathology was observed at ≥≈100 mg/kg-day of chlorobenzene (Hazleton Laboratories, 1967b; NTP, 1985; Irish, 1963). The kidney, bone marrow, thymus, and spleen were affected by treatment at higher chlorobenzene doses (NTP, 1985). The available data indicate that the developing fetus is not a sensitive target of orally administered chlorobenzene (IBT, 1977). Although no reproductive toxicity data from oral studies were located, the available inhalation data indicate that reproductive toxicity is not the most sensitive toxicological endpoint for chlorobenzene toxicity (Nair et al., 1987). Effects on immune system tissues were observed in the study conducted by NTP (1985); however, these

effects were only observed at substantially higher doses than those that induced liver toxicity. An inhalation exposure immune function assay indicated that the immune system is not likely a sensitive indicator of chlorobenzene toxicity (Aranyi et al., 1986). No neurotoxicity studies using oral exposure were located. Inhalation data appear to indicate that neurotoxicity data could be a sensitive endpoint of chlorobenzene toxicity in humans (Rosenbaum et al., 1947; Tarkhova, 1965; Ogata et al., 1991; Girard et al., 1969; and Syrovadko and Malysheva, 1977), although none of these studies were sufficient to definitively conclude that chlorobenzene causes adverse effects on the nervous system.

The 13-week study in dogs (Hazleton Laboratories, 1967a) was chosen as the basis for the subchronic RfD because this study demonstrated that the dog is the most sensitive species that has been evaluated in subchronic studies. In this study, chlorobenzene was administered to male and female dogs (4 per sex and dose) in gelatin capsules containing 0, 27.5, 55.0, 275 mg/kg-day of chlorobenzene 5 days per week for 13 weeks (duration-adjusted doses of 0, 19.6, 39.3 or 196.4 mg/kg-day). This study revealed treatment-related effects in the liver, kidneys, GI tract, and bone marrow at 196.4 mg/kg-day. At 39.3 mg/kg-day, effects on the liver (slight bile duct proliferation, slight swelling and vacuolation and leukocytic infiltration) and kidneys (swelling of tubular epithelium and variations in cellularity) were observed. No effects were observed in dogs administered 19.6 mg/kg-day chlorobenzene. Although none of the increased incidences at 39.3 mg/kg-day were significantly greater than controls, the study used only 8 dogs (4 per sex) per dose and the small number of animals resulted in low power of statistical analysis to detect a change. The study did show a clear increase in the incidence and severity of liver and bile duct hyperplasia with increasing dose. Therefore, the marginal increase in liver lesions and bile duct hyperplasia observed at 39.3 mg/kg-day was considered related to chlorobenzene treatment. This study, then, identified a NOAEL of 19.6 mg/kg-day and a LOAEL of 39.3 mg/kg-day for liver and bile duct hyperplasia.

The provisional **subchronic RfD of 7E-2 mg/kg-day** is derived from the NOAEL of 19.6 mg/kg-day by applying an uncertainty factor of 300 (10 to extrapolate from dogs to humans, 10 to protect sensitive subpopulations, and 3 for database deficiencies, including the lack of reproductive and neurological oral toxicity studies), as follows:

subchronic p-RfD	=	NOAEL ÷ UF
	=	19.6 mg/kg-day ÷ 300
	=	0.07 or 7E-2 mg/kg-day

Confidence in the principal study is medium. This study demonstrated a progression of effects with increasing dose, enabling identification of both a NOAEL and a LOAEL. However, the study was limited by small group sizes, lack of statistical analysis and only marginally adequate reporting of results. Confidence in the database is medium. Supporting oral toxicity data are available, but reproductive effects have been studied only by inhalation exposure, and neurotoxicity, which has been identified as a potential effect of chlorobenzene in humans exposed by inhalation, has not been systematically studied by any route. Medium confidence in the p-RfD follows.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR CHLOROBENZENE

Human data suggest that the nervous system may be a target for chlorobenzene toxicity (Rosenbaum et al., 1947; Girard et al., 1969; Tarkhova, 1965; Ogata et al., 1991). Headaches and drowsiness have been reported by workers and experimental subjects (Rosenbaum et al., 1947; Girard et al., 1969; Ogata et al., 1991), and tingling, numbness, and stiffness of the extremities have been observed in workers (Rosenbaum et al., 1947). However, none of these studies reported control data for these effects and the workers may have been exposed to other chemicals in addition to chlorobenzene. Thus, the observations may not have been related to chlorobenzene exposure. Tarkhova (1965) found alterations in the EEG pattern in response to rapid light flashes in humans exposed at 0.2 mg/m^3; however, the toxicological significance of these alterations is not known. Tarkhova (1965) also reported effects of unclear relevance (changes in the conduction speeds of nerve impulses to sets of flexor and extensor muscles) in rats exposed to chlorobenzene at 1.0 mg/m^3 for 60 days. The reliability of these data is uncertain because these effects have not been confirmed by other studies. Taken together, the data suggest that chlorobenzene may affect the nervous system. However, none of the data are adequate for use in risk assessment, either because the effects cannot be definitively attributed to chlorobenzene exposure, only single exposures were used, or the toxicological relevance of the effects is not clear.

The available animal data indicate that the liver and kidneys are the most sensitive target organs for chlorobenzene toxicity. Liver effects included increased weight, hepatocellular hypertrophy, fatty change, and other unspecified microscopic lesions (IBT, 1979; Nair et al., 1987; Dilley, 1977; Irish, 1963; Zub, 1978). Kidney effects included increased weights, cytoplasmic vacuolation, tubule dilation, inflammation of the interstitial cells, and regeneration of the epithelium in male rats (IBT, 1979; Irish, 1963; Nair et al., 1987; Dilley, 1977). The NOAEL and LOAEL for both liver and kidney effects were 50 and 150 ppm (230 and 691 mg/m^3, respectively) in the only adequately conducted and reported study (Nair et al., 1987). Kidney lesions have only been reported in male rats (or rats of unspecified sex) in the available inhalation studies, which suggests that the observed kidney effects may be related to alpha-$_{2u}$-globulin accumulation, a male rat-specific effect that is not predictive for health effects in humans (U.S. EPA, 1991b). Such an effect is known for other chlorinated benzene compounds (WHO, 1991). However, there does not appear to be sufficient evidence to attribute the kidney lesions observed by Nair et al. (1987) to alpha-$_{2u}$-globulin accumulation. Although the lesions were consistent with those associated with alpha-$_{2u}$-globulin, Nair et al. (1987) did not test for the presence of alpha-$_{2u}$-globulin directly. Chlorobenzene produced kidney lesions, including tubule dilation, vacuolation, and leukocytic infiltration, in dogs treated by oral exposure (Hazleton Laboratories, 1967a). NTP (1985) reported kidney effects in male and female mice (tubular necrosis) and male and female rats (degeneration and necrosis of the proximal tubule) in oral subchronic studies on chlorobenzene. The absence of kidney lesions in females (Nair et al., 1987) may reflect general lower sensitivity of females to chlorobenzene toxicity, as liver lesions were also observed only in males in this study. For these reasons, there is insufficient evidence to attribute the kidney lesions observed in this study to alpha-$_{2u}$-globulin accumulation, and the kidney effects are considered potentially relevant to human health risk assessment.

The testis was also identified as a target for chlorobenzene in male rats. Possible effects on the testes were observed in male rats exposed to chlorobenzene at 150 or 450 ppm (Nair et al., 1987). However, it does not appear that the testes are as sensitive a target as the liver and kidney because the incidence of the testicular lesions was only marginally increased in rats exposed at chlorobenzene concentrations that induced significant increases in the incidences of animals with microscopic liver and kidney lesions, and because reproductive performance was not affected.

Other studies suggested that the blood may be a potential target for chlorobenzene. Effects on the blood were observed by Zub (1978), who reported slight leukopenia and lymphocytosis in mice exposed to 100 mg/m^3 for 3 months. Dilley (1977) reported microcytic anemia in rats exposed at $\geq$ 336 mg/m^3. However, neither of these studies was adequate to base a definitive conclusion regarding effects on the blood, either because sufficient detail was not available to allow for an independent evaluation of study adequacy (Zub, 1978) or because the animals were sick during exposure (Dilley, 1977). Anemia was also reported in workers potentially exposed to unspecified concentrations of chlorobenzene; however, the workers were also exposed to other chemicals (Girard et al., 1969). Clear effects on the blood were not observed in dogs exposed to chlorobenzene for 6 months (IBT, 1979; Roloff, 1980) and blood effects have not been consistently reported in chlorobenzene exposed animals in subchronic studies; therefore, the data suggest that the blood is not likely a sensitive indicator of chlorobenzene toxicity.

Developmental toxicity studies in two species were located, which indicate that chlorobenzene is not a developmental toxicant (John et al., 1984). In a 2-generation reproductive toxicity study in rats (Nair et al., 1987), marginal increases in testicular lesions were associated with chlorobenzene exposure at concentrations that induced significant increases in the incidences of microscopic liver and kidney lesions. Reproductive impairment was not observed at any concentration. Therefore, it does not appear that reproductive toxicity is a sensitive endpoint for chlorobenzene toxicity.

Although a number of subchronic inhalation studies in animals were located (IBT, 1979; Roloff, 1980; Dilley, 1977; Irish, 1963), none of the these studies were considered adequate for RfC derivation for the following reasons: a clear LOAEL was not established (combined data from IBT, 1979 and Roloff, 1980); infection occurred in the test animals during exposure (Dilley, 1977); sufficient detail on the experimental design and results were not reported (Irish, 1963; Zub, 1978); or only one concentration was used (Zub, 1978). The only available study suitable for RfC derivation was the 2-generation study conducted by Nair et al. (1987). In this study, Sprague-Dawley rats (30 per sex and dose) were exposed to chlorobenzene (>99% pure) in a dynamic air chamber at target concentrations of 0, 50, 150, or 450 ppm (0, 230, 691, or 2072 mg/m^3) for 10 weeks before mating, then during mating, gestation, and lactation. Their offspring (F_1 rats) were exposed for 11 weeks beginning 1 week after weaning. Clear treatment-related effects were observed in the kidneys and liver of chlorobenzene exposed rats, and possible effects on the testes were observed. Kidney effects included increased weights, tubule dilation, inflammation of the interstitial cells, and regeneration of the epithelium in male rats; liver effects included increased organ weight and hepatocellular hypertrophy. The NOAEL and LOAEL for these effects was 50 and 150 ppm (230 and 691 mg/m^3, respectively). A marginal increase in the incidence of degeneration of the germinal epithelium was also observed at 150 ppm.

In order to derive the point of departure for derivation of the RfC, the LED_{10} (lower bound on dose estimated to produce a 10% increase in the extra risk of the modeled effects over background) was estimated for all kidney and liver lesions reported by Nair et al. (1987) using the U.S. EPA (2000) benchmark dose methodology. A 10% response level was modeled, as recommended for dichotomous endpoints by U.S. EPA (2000). The sensitivity of the study does not appear to warrant the use of a different response level (e.g., 1% or 5%). All available models for dichotomous data in the EPA Benchmark Dose Software (version 1.3.2) were fit to the incidence data for all treatment-related kidney and liver lesions observed in Nair et al. (1987) (incidence data reported in Table 7 below). Because each of the lesions were considered potentially relevant in human health risk assessment, the lesion that resulted in the lowest LED_{10} that was adequately described by modeling was chosen as the point of departure for the RfC. As illustrated in Table 7, renal tubular dilation resulted in the lowest LED_{10}. Tubular dilation can be caused by alpha-$_{2u}$-globulin accumulation in male rats. However, tubular dilation was observed in dogs orally administered chlorobenzene, and tubular necrosis, vacuolation, and/or regeneration was observed in male and female rats and mice orally administered chlorobenzene for 13 weeks. Therefore, tubular dilation is not necessarily a result of alpha-$_{2u}$-globulin accumulation and is potentially relevant to human health risk assessment; it was chosen as the point of departure for RfC derivation.

The dichotomous models estimated concentrations between 17 and 125 ppm associated with a 10% extra risk (ED_{10}) for tubular dilation (Table 8). As assessed by Akaike's Information Criterion (AIC), the best fitting models were the gamma, quantal linear, and Weibull models. Each of these models calculated ED_{10} values of 53.8 ppm and a lower 95% confidence interval (LED_{10}) of 39.7 ppm. Therefore, 39.7 ppm was selected as the point of departure to derive the p-RfC.

The LED_{10} of 39.7 ppm (183 mg/m^3) was converted to a human equivalent concentration using the following equations (U.S. EPA, 1994b):

$$LED_{10\ ADJ} = LED_{10} \times \text{duration adjustment}$$
$$LED_{10\ ADJ} = 183\ mg/m^3 \times 6\ hours/24\ hours \times 7\ days/7\ days$$
$$LED_{10\ ADJ} = 46\ mg/m^3$$

$$LED_{10\ HEC} = LED_{10\ ADJ} \times L_R/L_H$$

where,

L_R/L_H = rat to human blood:air partition coefficient ratio

L_R/L_H = default ratio of 1, because L_R (59.4; Gargas et al., 1989) is greater than L_H (30.0; Gargas et al., 1989)

$$LED_{10\ HEC} = 46\ mg/m^3 \times 1$$
$$LED_{10\ HEC} = 46\ mg/m^3$$

Table 7. LED_{10} Values Calculated for Chlorobenzene Based on Liver and Kidney Lesions in Nair et al. (1987)[a]

Organ, Lesion	Generation	Concentration (ppm)				LED_{10} (ppm)
		0	*50*	*150*	*450*	
Liver, hepatocellular hypertrophy	F_0	0/30[b]	0/30	5/30[c]	14/30[d]	97.3
	F_1	2/30	0/30	3/30	7/30	NA
Kidney, tubular dilation/eosinophilic material (unilateral or bilateral)	F_0	0/30	4/30	6/30[c]	18/30[d]	39.7
	F_1	8/30	7/30	14/30	22/30[d]	55.0
Kidney, chronic interstitial nephritis (unilateral or bilateral)	F_0	1/30	2/30	7/30[c]	10/30[d]	55.9
	F_1	1/30	3/30	7/30[c]	11/30[d]	49.5
Kidney, foci of regenerative epithelium (unilateral or bilateral)	F_0	0/30	1/30	5/30[c]	8/30[d]	73.0
	F_1	1/30	0/30	5/30	11/30[d]	116.7

[a] statistical analysis (Fisher Exact test) performed for this review and not by original investigators
[b] number of animals with lesion/total number of animals exposed
[c] statistically significant ($p \leq 0.05$)
[d] statistically significant ($p \leq 0.01$)
NA not assessed because statistical significance was not observed at any concentration

Table 8. ED_{10}, LED_{10}, and Selected Goodness of Fit Parameters from Modeled Incidence of Tubular Dilation/Eosinophilic Material (Unilateral or bilateral) Observed in Adult Male Rats Exposed to Chlorobenzene via Inhalation (Nair et al., 1987)

MODEL	ED_{10} (ppm)	LED_{10} (ppm)	χ^2 statistic	AIC
Gamma[a]	53.8	39.7	0.786	97.0
Quantal linear	53.8	39.7	0.786	97.0
Weibull[a]	53.8	39.7	0.786	97.0
Multi-stage[b]	56.6	39.8	0.586	99.0
Log-logistic[c]	51.33	17.0	0.501	99.4
Log-probit[c]	96.3	66.1	0.137	102.4
Probit	131.5	103.5	0.230	102.5
Logistic	143.4	111.6	0.210	102.9
Quantal quadratic	152.7	125.2	0.133	103.7

[a] Restrict power ≥ 1
[b] Restrict betas ≥ 0, Degree of polynomial = 2
[c] Slope restricted to >1

The $LED_{10\ HEC}$ of 46 mg/m^3 was divided by an uncertainty factor of 100 (3 to account for interspecies extrapolation using dosimetric adjustments, 10 to protect sensitive subpopulations, and 3 for database uncertainties [including the lack of adequate neurotoxicity data and the absence of a study that examined the entire respiratory tract]) to yield a provisional **subchronic RfC of 5E-1 mg/m^3**, as follows:

$$\text{subchronic p-RfC} = LED_{10\ HEC} \div UF$$
$$= 46\ \text{mg/m}^3 \div 100$$
$$= 0.5 \text{ or } 5\text{E-}1\ \text{mg/m}^3$$

Because no chronic inhalation toxicity studies were located in the literature, an additional subchronic-to-chronic uncertainty factor of 10 was applied to the provisional subchronic RfC to derive the provisional **chronic RfC of 5E-2 mg/m^3**, as follows:

$$\text{p-RfC} = \text{subchronic p-RfC} \div UF$$
$$= 5\text{E-}1\ \text{mg/m}^3 \div 10$$
$$= 5\text{E-}2\ \text{mg/m}^3$$

One area of uncertainty in the inhalation toxicity database for chlorobenzene is the lack of a study in which the entire respiratory tract was examined. None of the studies discussed in this issue paper examined the upper respiratory tract. Dilley (1977) examined the lungs, but the high incidence of chronic respiratory disease observed in the controls and chlorobenzene-exposed animals limited the ability of this study to detect chlorobenzene-related lung effects.

Data reported by Irish (1963) indicate that the lungs are not more sensitive than the liver or kidneys to chlorobenzene effects, although data from this study were not adequately reported and, therefore, cannot be independently assessed. In the Ogata et al. (1991) human study, none of the subjects complained of nose or eye irritation, although one of the subjects did complain of a sore throat following a 7-hour exposure to 60.2 ppm (277 mg/m^3). Another area of uncertainty is the lack of neurological testing. The available human data (Ogata et al., 1991) suggest that the nervous system may be a sensitive target of chlorobenzene toxicity. Headaches and drowsiness were reported by experimental subjects during exposure to 60.2 ppm (277 mg/m^3). The subchronic and chronic RfCs that were derived from Nair et al. (1987) (0.5 and 0.05 mg/m^3, respectively) are substantially lower than concentrations associated with these effects. Although Tarkhova (1965) reported changes in electroencephalographic (EEG) patterns in response to light flashes in 2/4 human subjects exposed at 0.2 mg/m^3, the toxicological relevance of this effect is not clear, and the reliability of these data is uncertain. Other studies have reported potential neurological effects in exposed humans (Rosenbaum et al., 1947; Girard et al., 1969); however, there is some uncertainty whether these effects were related to chlorobenzene exposure because neither of these studies reported data from unexposed controls. None of the repeated-dose animal studies observed overt signs of neurological effects. Tarkhova (1965) reported potential effects in rats at 0.2 ppm (1 mg/m^3); however, the toxicological significance of the reported effect (changes in the conduction speeds of nerve impulses to sets of flexor and extensor muscles on Day 39) is not clear, and these results have not been confirmed by other studies.

Confidence in the principal study (Nair et al. 1987) is high. It is a well designed two-generation study examining relevant endpoints with an adequate number of animals. Confidence in the database is low. As discussed above, the database lacks a study that adequately examined the entire respiratory tract, and also lacks an adequate neurotoxicity study. Because the available data suggest that neurotoxicity may be a sensitive toxicological endpoint for chlorobenzene, confidence in the provisional chronic and subchronic RfC is low.

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