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Provisional Peer-Reviewed Toxicity Values for

Hexachlorobenzene
(CASRN 118-74-1)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower bound 95% confidence interval
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
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
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 reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR HEXACHLOROBENZENE (CASRN 118-74-1)

BACKGROUND

HISTORY

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA) 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 (PPRTVs) 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 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 a panel of six EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram 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 5-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 documents 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 Resource Conservation and Recovery Act (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 document 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, 2009) contains an RfD for hexachlorobenzene (see Figure 1 for structure of hexachlorobenzene) of 8×10^{-4} mg/kg-day, based on a NOAEL of 0.08 mg/kg-day (estimated from a dietary concentration of 1.6 ppm) for liver effects in rats from a study by Arnold et al. (1985) and an uncertainty factor (UF) of 100. The study by Arnold et al. (1985) identifies a LOAEL of 0.29 mg/kg-day (estimated from a dietary concentration of 8 ppm). IRIS cited the *Health Assessment Document* (HAD) *for Chlorinated Benzenes* (U.S. EPA 1985) and the *Drinking Water Criteria Document* (DWCD) *for Hexachlorobenzene* (U.S. EPA, 1988a) as source documents for the existing assessment. The IRIS RfD of 8×10^{-4} mg/kg-day is also included on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) and the HEAST (U.S. EPA, 1997). The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1994, 1991) included the previously mentioned HAD and DWCD documents as well as a 1984 Health Effects Assessment (HEA) for hexachlorobenzene (U.S. EPA, 1984) that did not derive toxicity values.

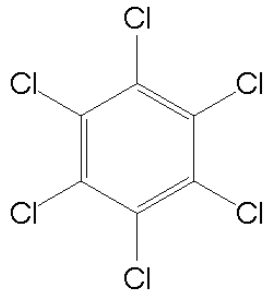

Figure 1. Chemical Structure of Hexachlorobenzene

ATSDR published a toxicological profile for hexachlorobenzene (ATSDR, 2002) that includes

- an acute-duration oral minimal risk level (MRL) of 0.008 mg/kg-day based on a developmental LOAEL of 2.5 mg/kg-day for hyperactivity in offspring of rats treated prior to mating (Goldey and Taylor, 1992) and a UF of 300;
- an intermediate-duration oral MRL of 1×10^{-4} mg/kg-day based on a LOAEL of 0.01 mg/kg-day for minimal ovarian effects in monkeys (Bourque et al., 1995; Jarrell et al., 1993) and a UF of 90; and
- a chronic-duration oral MRL of 5×10^{-5} mg/kg-day based on a LOAEL of 0.016 mg/kg-day for hepatic effects in male F1 rats in a multigeneration study (Arnold et al., 1985) and a UF of 300.

The World Health Organization (WHO, 1997) published a criteria document for hexachlorobenzene in which a tolerable daily intake (TDI) of 1.7×10^{-4} mg/kg-day was derived on the basis of the lowest reported NOEL of 0.05 mg/kg-day (with hepatic effects at higher doses in pigs and rats) and a UF of 300. The National Toxicology Program (NTP) has assessed the oral toxicity of hexachlorobenzene in a 13-week gavage study with female Sprague-Dawley rats (NTP, 2001) and a 6-month oral continuous breeding study with male and female Sprague-Dawley rats (Wolfe and Pepperl, 2005).

No RfC for hexachlorobenzene is available on the IRIS database (U.S. EPA, 2009); lack of data precluded derivation of an RfC. Likewise, ATSDR (2002) did not derive inhalation MRLs due to the lack of human or animal studies. The American Conference of Governmental Industrial Hygienists (ACGIH, 2008) lists a threshold limit value (TLV) for hexachlorobenzene of 0.002 mg/m^3 as an 8-hour time-weighted average (TWA) to protect against porphyria, skin damage, and central nervous system impairment. This assessment was based on oral exposure data because of the lack of human or animal inhalation data. The National Institute of Occupational Safety and Health (NIOSH, 2009) and the Occupational Safety and Health Administration (OSHA, 2009) do not report permissible exposure limits for hexachlorobenzene.

A cancer assessment for hexachlorobenzene is available on IRIS (U.S. EPA, 2009). A weight-of-evidence (WOE) of classification of B2 (*Probable Human Carcinogen*) was assigned on the basis of sufficient animal evidence (i.e., increased incidence of liver, thyroid, and kidney tumors in mice, rats, and hamsters) and inadequate human evidence. The existing cancer assessment was conducted under the EPA (1986) *Guidelines for Carcinogen Assessment*, and was subsequently revised in 1998. Hexachlorobenzene has not been evaluated under the EPA (2005) *Guidelines for Carcinogen Risk Assessment.* An oral slope factor (OSF) of 1.6 per (mg/kg-day) was derived using a data set for hepatocellular carcinomas in female Sprague-Dawley rats. In deriving the final OSF, EPA (2009) considered 14 different data sets from three species, four studies, and various tumor endpoints. The OSFs derived from these data sets all fell within a range of approximately one order of magnitude (0.083–1.7). An inhalation unit risk (IUR) of 4.6×10^{-4} per ($\mu g/m^3$) was derived by route-to-route extrapolation from the oral data set. IRIS cited the EPA (1985) HAD for chlorinated benzenes and the EPA (1988a) DWCD for hexachlorobenzene as source documents for the cancer assessment.

The International Agency for Research on Cancer (IARC) has evaluated the carcinogenicity of hexachlorobenzene (IARC, 2001, 1987, 1979) and assigned a WOE

classification of 2B (*Possibly Carcinogenic to Humans*). This classification is based on inadequate evidence in humans and sufficient evidence in animals. Hexachlorobenzene is included in the *11th Report on Carcinogens* (NTP, 2005) and is classified as *Reasonably Anticipated to be a Human Carcinogen*. ACGIH (2008) gives a cancer designation of A3 (*Confirmed Animal Carcinogen with Unknown Relevance to Humans*). WHO (1997) estimated a tumorigenic dose associated with a 5% excess incidence of tumors (TD_5) of 0.81 mg/kg-day based on the incidence of neoplastic nodules of the liver in female F1 rats in the study reported by Arnold et al. (1985). Citing insufficient mechanistic data, WHO (1997) applied a UF of 5,000 to the TD_5 to derive a guidance value of 1.6×10^{-4} mg/kg-day.

CalEPA (2003) has established a Public Health Goal (PHG) for hexachlorobenzene of 0.03 ppb (0.03 µg/L) in drinking water. The PHG is based on carcinogenic effects in animals and is estimated from the observed incidence of adrenal pheochromocytomas and hepatocarcinomas in female rats exposed to dietary hexachlorobenzene (Arnold and Krewski, 1988, Lambrecht et al., 1983a,b). CalEPA (2009) lists an IUR value of 5.1×10^{-4} $(\mu g/m^3)^{-1}$ and an OSF of 1.8 per (mg/kg-day) for hexachlorobenzene on the Air Toxics Hot Spot Program Risk Assessment Guidelines.

Because IRIS contains both a chronic oral RfD and cancer assessment for hexachlorobenzene (U.S. EPA, 2009), this PPRTV document aims only to provide subchronic p-RfD derivation and subchronic and chronic p-RfC development. Given the size of the toxicological database for hexachlorobenzene, earlier assessments for hexachlorobenzene, including the IRIS assessment (last revised in 1991) and the ATSDR (2002) toxicological profile were consulted to identify information relevant to this PPRTV. Literature searches were conducted from January, 2002 through September 3, 2010 for studies published since the ATSDR (2002) toxicological profile that might be relevant to the derivation of provisional toxicity values for hexachlorobenzene. Databases searched included MEDLINE, TOXLINE (with the National Technical Information Service [NTIS]), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months).

REVIEW OF PERTINENT DATA

HUMAN STUDIES

A number of recent studies have examined associations between hexachlorobenzene in human biological fluids and various health outcomes. For example, recent studies have correlated the concentration of hexachlorobenzene in umbilical cord serum at birth with decreased gestational length (Fenster et al., 2006), poor social competence (Ribas-Fito et al., 2007), increased body mass index and weight during childhood (Smink et al., 2008), and increased urinary coproporphyrins in childhood (Sunyer et al., 2008). There are also numerous studies that investigate correlations between serum or tissue concentrations of hexachlorobenzene and various disease states in adult humans. However, none of the available studies can be used for quantitative risk assessment due to the confounding effects of exposure to other organochlorines and due to the lack of exposure information.

Dietary exposure to hexachlorobenzene by ingestion of bread made from contaminated grain resulted in an outbreak of porphyria cutanea tarda and mixed porphyria in Turkey during 1955–1959 (Peters et al., 1987; Gocman et al., 1986). Exposed adults and older children experienced effects that included muscle weakness, loss of appetite, photosensitivity with formation of bullae (blisters) and milia (whiteheads) on sun-exposed areas, hyperpigmentation, hypertrichosis (excessive hair growth), orthopedic abnormalities including osteoporosis and arthritis, fragile skin, goiter, hepatomegaly, and porphyrinuria. Thirty years after exposure, many of these effects were still prevalent in the exposed population. Almost all children born to mothers who consumed hexachlorobenzene-contaminated bread during pregnancy died within 1 year, after exhibiting weakness, convulsions, and characteristic skin lesions. In addition to in utero exposure, some of these children were presumed to have been exposed postnatally by lactational transfer. Of 188 pregnancies that occurred during 1977–1981 in 42 women who were exposed as children or young adults, there were 15 fetal deaths (13 miscarriages and 2 stillbirths) and 173 live births (Peters et al., 1987). The human data are not suitable for use in quantitative risk assessment because actual exposure doses and durations are not known. However, the original investigators of the epidemic estimated that the amount of hexachlorobenzene ingested by the victims was approximately 0.05–0.2 g/day (0.7–2.9 mg/kg-day for a 70-kg person) "for a relatively long period before the skin manifestations of the disease became apparent" (Cam and Nigogosyan, 1963).

ANIMAL STUDIES

Oral Exposure

The database for oral toxicity of hexachlorobenzene (HCB) is extensive (see ATSDR, 2002). Because this review is limited to an assessment of oral data pertinent to the derivation of a subchronic p-RfD, and given the size of the toxicological database, the text that follows summarizes only studies that might be useful in deriving a subchronic p-RfD. The studies considered potentially relevant to subchronic p-RfD derivation included (1) the principal study (Arnold et al., 1985) used to derive the chronic RfD on IRIS (U.S. EPA, 2009) and the chronic-duration oral MRL (ATSDR, 2002) because this study included a subchronic-duration exposure component (F0 generation); (2) the key studies used by ATSDR (2002) to derive the intermediate-duration oral MRL; and (3) any short-term, subchronic, developmental, and reproductive toxicity studies published since the ATSDR (2002) toxicological profile.

Principal Study for Chronic RfD (U.S. EPA, 2009) and Chronic Oral MRL (ATSDR, 2002)

Arnold et al. (1985)—In a single-generation reproductive toxicity study that included chronic-duration exposure of offspring, Sprague-Dawley rats (64–66/sex/group) were fed diets containing 0, 0.32, 1.6, 8.0, or 40.0 ppm of hexachlorobenzene (purity not specified) dissolved in corn oil for 90 days (Arnold et al., 1985). Doses are estimated to be 0, 0.03, 0.14, 0.69, and 3.4 mg/kg-day for males and 0, 0.03, 0.16, 0.78, and 3.9 mg/kg-day for females, based on EPA (1988b) reference values for body weight (male = 0.267 kg, female = 0.204 kg) and food intake (male = 0.023 kg/day, female = 0.020 kg/day) for subchronic-duration exposure in Sprague-Dawley rats. Individual rat body weight and cage-group feed consumption were measured weekly. Animals were mated after 90 days of treatment; it is unclear whether animals were also exposed to hexachlorobenzene during gestation and lactation. The F0 parental animals were sacrificed after the lactation period. Hematological variables (not specified), gross pathology, organ weights (i.e., liver, kidneys, heart, spleen, testes or ovaries, adrenals, and brain), and histopathological examination of liver, kidneys, and grossly abnormal areas of

dermal, supportive,[1] or skeletal tissues were used to assess F0 parental toxicity. Fertility, gestation, viability, and lactation from the F0 mating were assessed as measures of reproductive toxicity.

In the F0 parental animals, body weight and feed consumption were not affected by treatment (data not shown). Although sporadic statistical differences in hematological parameters were seen among the F0 males (hematocrit values in the 0.32-ppm group, monocyte number in the 40-ppm group, and bone-marrow myeloid and erythroid series results for the 1.6-and 8.0-ppm groups [no further information provided]), the study authors indicated that their occurrence was not dose-related and probably not due to hexachlorobenzene exposure. Also in F0 males, significant ($p < 0.05$) organ-weight increases were reported in the 8-ppm (i.e., absolute and relative heart and brain weights and relative liver weights) and 40-ppm (i.e., absolute heart and relative liver weights) exposure groups (data not shown). In the F0 females, no treatment effects on organ weights or hematology were observed. No differences in gross or microscopic pathology were noted between control and hexachlorobenzene-treated F0 animals. Treatment did not alter the fertility, gestation, or lactation indices, but the viability index was significantly reduced at 40 ppm (data not shown), indicating increased mortality of pups between birth and Postnatal Day (PND) 4 at this dose. The NOAEL for parental toxicity in the F0 generation (exposed subchronically) is 1.6 ppm (0.14 mg/kg-day); the LOAEL is 8 ppm (0.69 mg/kg-day) based on increased organ weights. The NOAEL for reproductive toxicity is 8 ppm (0.69 mg/kg-day); the LOAEL based on decreased viability between birth and PND 4 is 40 ppm (3.4 mg/kg-day).

In the chronic-duration exposure portion of the study, the F1 animals were reduced to 50/sex/dose group at weaning and were fed their parents' diets for the remainder of their lifetime (130 weeks). However, F1 animals were not bred; thus, there are no reproductive data for this generation. The chronic-duration study was used to derive the RfD on the IRIS database; EPA determined that 1.6 ppm (0.08 mg/kg-day) was a NOAEL and 8 ppm (0.3 mg/kg-day) was a LOAEL for increased hepatic centrilobular basophilic chromogenesis (U.S. EPA, 2009).

Critical Studies for ATSDR Intermediate-Duration Oral MRL

Jarrell et al. (1993); Foster et al. (1992); Babineau et al. (1991); Sims et al. (1991)—Ovarian function and histopathology were studied in female Cynomolgus monkeys (captive bred, approximately 5 years of age, four/dose group) that were given hexachlorobenzene (purity not stated) mixed with sucrose in gelatin capsules at doses of 0, 0.1, 1, or 10 mg/kg-day for 90 days (Jarrell et al., 1993; Foster et al., 1992; Babineau et al., 1991; Sims et al., 1991). During the first menstrual period following the end of dosing, the monkeys were treated with hormones to induce superovulation. The hormone treatments consisted of six daily injections of human menopausal gonadotropin (a mixture containing follicle stimulating [FSH] and luteinizing hormones [LHs]) beginning on Menstrual Day 2 or 3, followed by an injection of human chorionic gonadotropin (HCG) on Day 9 or 10 of the cycle. Laparotomy for follicle aspiration and recovery of oocytes and granulosa cells was performed 35 hours after the HCG treatment (i.e., within 2 hours of the estimated time of ovulation). The animals were then sacrificed, and both ovaries were removed from each monkey for examination by thin-section histological techniques on one ovary and transmission electron microscopy (TEM) on the other ovary. Recovered oocytes were used for in vitro fertilization, and granulosa cells were incubated with HCG to measure progesterone (by

[1]This is the term used by the study authors; the tissues included in this category are not specified.

radioimmunoassay) in the supernatant. Menstrual cycle length and duration, and circulating levels of estradiol (E_2) and progesterone (P_4) during the cycle were assessed based on daily vaginal swabs and serum analyses. Concentrations of hexachlorobenzene were measured in serum (weekly) and postmortem tissues (e.g., liver, kidney, brain, fat). Other study endpoints included weekly measurements of body weight, biweekly collection of urine and blood samples for serum gamma glutamyl transferase (GGT), aspartate aminotransferase (AST, or serum glutamic-oxaloacetic transaminase [SGOT]), sorbital dehydrogenase (SDH), urinary d-glucaric acid and porphyrins, and serum HCB levels, and terminal measurements of organ weights (unspecified except for ovary) and liver histology.

Specific endpoints were reported in different publications as follows: systemic toxicity, hexachlorobenzene tissue concentrations, primordial follicle histology, and oocyte function (Jarrell et al., 1993); serum oestradiol and progesterone concentrations and menstrual cycling (Foster et al., 1992); morphological effects on the ovarian surface epithelium as assessed by thin section light microscopy (Sims et al., 1991); and ultrastructural changes in the ovarian surface epithelium as assessed by TEM (Babineau et al., 1991). Effects reported in these studies are discussed below.

Serum and tissue concentrations of hexachlorobenzene increased in a dose- and time-dependent manner, with the highest levels occurring in the tissues with the highest lipid content (Jarrell et al., 1993). No clinical signs of systemic toxicity, effects on weight gain, or changes in liver enzyme or urinary porphyrin levels were found in any of the monkeys, and response to in vitro fertilization was effective in all animals. Dose-related histopathological effects occurred in the liver at ≥1 mg/kg-day, including accentuated zonation, increased portal density, mid-zonal vacuolation, and intrahepatic cholestasis. Liver and adrenal weights (data not reported) were significantly increased at 10 mg/kg-day (Jarrell et al., 1993).

Hexachlorobenzene caused a statistically significant ($p < 0.05$) decrease in serum progesterone concentrations during the luteal phase of the menstrual cycle at ≥1 mg/kg-day (Foster et al., 1992). There were no exposure-related changes in serum progesterone during the follicular and periovulatory phases, serum estradiol levels, menstrual cycle length, or duration of menses. Although no statistical differences were found in mean cycle length, Foster et al. (1992) reported increased variability in cycle length at 10 mg/kg-day (ranged from 29–58 days compared with 28–36 days in controls).

A statistically significant ($p < 0.05$; see Table 1) decline in the total number of primordial follicles was observed at 10 mg/kg-day (Jarrell et al., 1993). Histological changes in the ovarian follicles occurred at all dose levels and increased in severity with increasing dose; changes included decreased distinctiveness of follicular nuclear and nucleolar membranes; increased granularity, density, and irregular shape of oocyte nuclei; increased vacuoles and aggregated lysosomes in oocyte cytoplasm; and pyknotic granulosa cells in antral and preantral follicles.

Table 1. Significant Effects of 90-Day Oral Exposure to Hexachlorobenzene on Serum Hexachlorobenzene Concentration and Ovarian Histology in Monkeys

Endpoint	Dose (mg/kg-day)			
	0	0.1	1.0	10.0
Number examined unless noted otherwise	4	4	4	4
Serum hexachlorobenzene (ppm)	0.01 ± 0.01[a, b]	0.16 ± 0.10	0.37 ± 0.06	1.31 ± 1.00
Number of primordial ovarian follicles	26,348 ± 9,860	19,473 ± 4,504	24,027 ± 3,717	8,737± 3,047[c]
Mean proportion of normally shaped (cuboidal) cells in surface epithelium (number of animals)	77.6 ± 5.2% (503 cells from four animals)	23.0 ± 7.2%[d] (375 cells from two animals)	11.8 ± 4.6%[d] (381 cells from three animals)	30.2 ± 5.7%[d] (454 cells from four animals)

[a]Mean ± SEM.
[b]Serum concentrations increased significantly in a dose-dependent manner, $p = 0.0005$.
[c]$p < 0.05$, significantly different from controls per analysis of variance and Duncan's multiple range test.
[d]Significantly different from controls per study authors, but *p*-value and test were not specified.

Sources: Jarrell et al. (1993); Sims et al. (1991).

TEM examination of the ovarian follicles confirmed the dose-related histological changes. Jarrell et al. (1993) reported the following changes: increased numbers of lysosomal elements in the ooplasm of the developing ova and irregularly arranged cells in the thecal layer at 0.1 mg/kg-day; loss of chromatin and pyknosis in the nucleus of the developing ovum, occasional necrosis in the ooplasm, and mild-to-moderate degenerative changes in follicular cells at 1 mg/kg-day; and vacuolated and necrotic ooplasm and loss of typical stratified arrangement of follicular cells at 10 mg/kg-day.

Light and TEM microscopy of the surface epithelium (SE) of the ovary also showed dose-related effects (Babineau et al., 1991; Sims et al., 1991). Dose-dependent proliferative and degenerative changes of increasing severity were noted at all dose levels, including increasingly severe and frequent changes in cell shape, from squamous and cuboidal to tall columnar. At 0.1 mg/kg-day, the SE lengthened from normally flat, cuboidal-shaped cells into narrow columnar cells, and some stratification (proliferation) and areas of degeneration were noted. Intracellular alterations occurred mainly in the columnar cells; nuclei were elongated and had migrated toward the apical surface, and cytoplasmic degradation was apparent as indicated by reduced number of organelles (except lysosomes). At 1 mg/kg-day, lipid accumulations concentrated within clusters of cells, cytoplasmic degeneration was observed in some affected cells including vacuolation and flattening of cells, and cellular necrosis and denuding were observed, although most of the SE remained intact. At 10 mg/kg-day, mixed cell morphology (normal, columnar, cuboidal, and squamous shapes) was observed interspersed with regions of cell death. In the cytoplasm, lipid inclusions were present, and frequent myelin-like bodies and irregular and swollen microvilli were observed. Separation of SE from the connective tissues was also apparent at this dose. Stratification of the SE occurred, and surface indentations accentuated by edema were observed. A quantitative analysis of the observed morphological changes as assessed by light microscopy indicated that the proportion of normal cells in the exposed groups was significantly ($p < 0.05$) lower than in the controls when cell shape was

measured (see Table 1) but not when lipid accumulation was used as a criterion (Sims et al., 1991).

Based on the multiple histological findings in the ovarian follicles and surface epithelium, 0.1 mg/kg-day (the lowest dose tested) is a LOAEL for these studies.

Bourque et al. (1995)—As a follow-up to the previous studies, a detailed ultrastructural study of ovarian follicles in hexachlorobenzene-exposed monkeys was conducted with the addition of a lower dose (Bourque et al., 1995). Female Cynomolgus monkeys (4/dose, 6−13 years of age) were administered hexachlorobenzene (purity not stated) mixed with glucose in gelatin capsules, daily, at doses of 0, 0.01, 0.1, 1.0, or 10 mg/kg-day for 13 weeks. Controls received only glucose. After the period of treatment, monkeys were given FSH and LH during Days 2 through 7 of the following menstrual period. On the eighth day of the cycle, HCG was given; an ovary from each monkey was subsequently removed 35–38 hours later. Ovaries were sectioned, and primordial, primary, and growing follicles from controls and each hexachlorobenzene treatment group were examined by TEM.

Ultrastructural changes were noted in the ovarian follicles of all hexachlorobenzene-exposed monkeys (Bourque et al., 1995). Incidences of the observed effects were not given; a narrative description of the increasing severity of effects follows. In control monkeys, the developing ova had normal mitochondria that were typically distributed, and follicular cells surrounding the ova were also described as normal in shape and content of their nuclei. In ova of monkeys treated with hexachlorobenzene at a dose of 0.01 mg/kg-day, the majority of mitochondria were condensed with swollen cristae; follicular cells were generally unaffected, but "a few cells" contained abnormal nuclei. In ova of monkeys treated with hexachlorobenzene at a dose of 0.1 mg/kg-day, the mitochondria contained coarsely granular matrices and/or exhibited irregular shapes; many of the follicular cells contained abnormal nuclei (infolding of the nuclear membrane). In ova of monkeys treated with hexachlorobenzene at a dose of 1 mg/kg-day, mitochondria were condensed and swollen; in addition, herniation of the ooplasm (suggesting rupture of the zona pellucida) was noted, along with abnormal nuclei in follicular cells and abnormal spaces between follicular cells. In ova of monkeys treated with hexachlorobenzene at a dose of 10 mg/kg-day, the mitochondrial changes were more severe (many had electron-lucent matrices); in follicular cells, the nuclear membrane was highly folded with deep indentations, and there was an abnormal amount of lipid in the cells. Further, the cells of the theca folliculi of the stroma were affected (deformed nuclei) only in monkeys treated with 10 mg/kg-day. The study authors concluded that a NOAEL was not defined (Bourque et al., 1995). The LOAEL for this study is 0.01 mg/kg-day based on degenerative changes in primary and growing ovarian follicles.

Foster et al. (1995)—Effects of hexachlorobenzene on ovarian steroidogenesis and menstrual cycle characteristics were investigated in groups of four feral female Cynomolgus monkeys (estimated 6–18 years of age) treated daily with capsules containing 0-, 0.1-, 1.0-, or 10-mg hexachlorobenzene/kg-day in glucose for 90 days (Foster et al., 1995). The 0.1- and 1.0-mg/kg-day dose levels were selected to provide circulating levels in the monkey equivalent to levels found in the general population and in people with occupational exposure to hexachlorobenzene. The dosing period was preceded by a 10-week acclimation phase, included three menstrual cycles, and was followed by ovulation induction with FSH and LH on Cycle

Days 2–7, ovulation stimulation with HCG on Cycle Day 8, and oophorectomy on Cycle Day 10 via laparotomy. Study endpoints included tissue levels of hexachlorobenzene (serum, fat, and follicular fluid), clinical signs, body weight, routine clinical chemistry and hematology indices, serum indicators of ovarian function (E_2, P_4, and inhibin [INH]), and menstrual cycle length and duration of menses. The menstrual cycle evaluations included both exposed-control group comparisons and comparisons in which the monkeys served as their own controls (i.e., third cycle during treatment compared with cycle preceding treatment).

Dose-related increases in concentrations of hexachlorobenzene in fat, serum, and follicular fluid were detected. No effects on clinical signs, hematologic parameters, body weight, or food and water consumption were observed. The level of hexachlorobenzene in the follicular fluid was increased at ≥1 mg/kg-day (not detected at 0 and 0.1 mg/kg-day) and statistically significantly ($p < 0.05$) correlated with follicle volume. Other exposure-related effects included alterations in menstrual cycle duration and ovarian function. Comparison of mean menstrual cycle lengths showed no significant differences in the treated and control groups during the pretreatment or treatment periods. Comparison of pretreatment and treatment values for each group, however, showed that cycle length was statistically significantly ($p < 0.05$) longer during the treatment period at 10 mg/kg-day compared with controls; the mean menstrual cycle lengths during treatment were 1.5, 2.5, 9.1, and 12.4 days longer than pretreatment values at 0, 0.1, 1, and 10 mg/kg-day, respectively. No treatment-related changes in mean length of the follicular and luteal phases were found using either method of comparison. An effect on ovarian function was indicated by statistically significantly ($p < 0.05$) reduced serum levels of estradiol during ovulation at 10 mg/kg-day, as determined by analysis of areas under time-concentration curves (AUCs) or exposed-control group comparisons of peak estradiol levels ($p = 0.03$, Dunnett's test). There was no difference in the number of oocytes recovered, volume of antral follicles, or number of corpora lutea (data not reported). The NOAEL for this study is 1 mg/kg-day. The LOAEL is 10 mg/kg-day for increased mean menstrual cycle length accompanied by reduced serum estradiol during ovulation.

Subchronic, Developmental, or Reproductive Toxicity Studies Published Since ATSDR (2002)

Long et al. (2004), Johnson et al. (2005)—Long et al. (2004) and Johnson et al. (2005, abstract only) reported selected findings from a subchronic-duration study of hexachlorobenzene conducted by the NTP. Draft body weight, survival, clinical observations, and neoplastic and nonneoplastic effect incidence data are available online,[2] but it is uncertain as to when the final data and/or full report may be posted. According to the available information, female Sprague-Dawley rats (10/dose) were administered hexachlorobenzene (purity >99%) in corn oil by gavage at doses of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, or 25 mg/kg-day, 5 days/week, for 13 weeks. Few details are available on toxicological evaluations. Long et al. (2004) reported that animals were observed twice daily for morbidity and mortality, and that, at terminal sacrifice, three transverse sections through the nose were excised at specific landmarks to facilitate examination of the right and left maxillary incisors and the right and left second molars. Sections were stained with hematoxylin and eosin and were evaluated by an NTP Pathology Working Group. In an abstract, Johnson et al. (2005) reported the following findings: increased mean body weight at 25 mg/kg-day but no treatment-related effect on survival; decreased total

[2]http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=shorttermbioassaydata.datasearch&chemical_name=Hexachlorobenzene&cas_no=118-74-1&study_no=C98004&study_length=13%20Weeks.

thyroxine (T_4) (≥10 mg/kg-day); decreased free T_4 (≥1 mg/kg-day); decreased triiodothyronine (T_3) (25 mg/kg-day); no effects on thyroid-stimulating hormone (TSH); dose-related increase in hepatic CYP1A1 (≥1 mg/kg-day), CYP2B (≥0.03 mg/kg-day), and CYP1A2 (≥0.3 mg/kg-day) and pulmonary CYP1A1 (25 mg/kg-day) activities; increased liver cell proliferation (≥10 mg/kg-day); increased absolute and relative organ weights (spleen, liver, and lung at doses ≥3, ≥10, and 25 mg/kg-day, respectively); hepatocellular hypertrophy (≥3 mg/kg-day); pulmonary focal interstitial fibrosis and histolytic infiltration (≥1mg/kg-day); mammary gland hyperplasia (≥10 mg/kg-day); thymic atrophy (≥10 mg/kg-day); dermal suppurative inflammation and ulceration (25 mg/kg-day); splenic hematopoietic cell proliferation (≥3 mg/kg-day); and lymphoid hyperplasia (≥10 mg/kg-day). The focus of the report by Long et al. (2004) was dental effects. Long et al. (2004) reported that maxillary incisor degeneration was observed at doses of ≥1 mg/kg-day. No evidence of thrombosis, hemorrhage, or vasculitis was noted by the study authors. Table 2 shows incidence and severity of incisor degeneration. Based on the available information, these studies appear to support a NOAEL of 0.3 mg/kg-day and a LOAEL of 1 mg/kg-day for pulmonary focal interstitial fibrosis and histolytic infiltration, maxillary incisor degeneration, and effects on thyroid hormones (decreased free T_4). These effect levels are considered tentative until the full NTP report or a publication providing the remaining data becomes available.

Table 2. Incidence and Severity of Incisor Degeneration in Female SD Rats Exposed Orally to Hexachlorobenzene for 13 Weeks

	Dose (mg/kg-day)							
Severity	**0**	**0.03**	**0.1**	**0.3**	**1**	**3**	**10**	**25**
Minimal	0	0	0	0	5	5	1	0
Mild	0	0	0	0	0	5	7	1
Moderate	0	0	0	0	0	0	2	9
Marked	0	0	0	0	0	0	0	0
Number examined	10	10	10	10	10	10	10	10

Source: Long et al. (2004).

Wolfe and Pepperl (2005)—Wolfe and Pepperl (2005) evaluated the reproductive toxicity of hexachlorobenzene in a continuous breeding study conducted for the NTP. The study report was not published and is not available on the NTP Web site, but is available through NTIS. The initial task of the study consisted of a dose-range finding experiment; eight/sex/group Sprague-Dawley rats were exposed to 1, 10, 100, or 1,000 µg/kg-day hexachlorobenzene (99% purity) by daily gavage for 1 week prior to mating and during mating, gestation, and through parturition to PND 1. Parental observations were limited to clinical signs of toxicity, body weight, and food and water consumption. Litter sizes were recorded, and pups were weighed and sexed. In addition, anogenital distance (AGD) of male and female pups was measured, and gross examination of the reproductive system and kidneys was performed.

As no systemic or reproductive effects were observed in the dose-range finding study, the doses were increased in the main study, in which groups of 20 male and 20 female Sprague-Dawley rats were administered doses of 0, 0.5, 2.5, or 12.5 mg/kg-day for two generations (Wolfe and Pepperl, 2005). The F0 adults were bred continuously to produce

F1a, F1b, and F1c pups; F1c pups were raised and bred continuously to produce F2a, F2b, and F2c pups. Males in the F0 generation were dosed from the initiation of the study, and females in the F0 generation were dosed from Study Day 15 until the day before necropsy (Study Days 161, 162, or 163). Parental body weights were measured regularly. A comprehensive assessment of reproductive and teratogenic endpoints was conducted on F1a and F1b litters, while reproductive system development and neurotoxicity endpoints were evaluated in F1c litters. In addition, one male and five females from the control and low-dose groups were sacrificed and necropsied at weaning; blood samples were also collected at this time for analysis of thyroid hormones. F2 litters were evaluated for litter size, numbers of live and dead pups, sex ratio, and AGD, but no other parameters. Major organs and those of the reproductive system were weighed and examined microscopically in F0 and F1 parents. Table 3 provides an outline of the tasks and evaluations performed in this study.

Table 3. Summary of Tasks and Evaluations in Reproductive Assessment by Continuous Breeding Protocol for Hexachlorobenzene

Task 1: Dose range-finding study			
Parental systemic	**Parental reproductive**	**Pups**	
Mortality	Pregnancy index	Litter observations[a]	
Body weight, food consumption, water consumption		Anogenital distance	
Task 2: Main study			
F0 systemic	**F0 reproductive**	**F1a and F1b pups**	**F1c pups**
Mortality	Sperm analysis	Litter observations[a]	Litter observations[a]
Body weight, food consumption	Vaginal cytology	Thyroid hormone levels[b]	Anogenital distance
Thyroid hormone levels[b]	Pregnancy index	Anogenital distance	Viability to weaning
Necropsy	Gestation	Visceral evaluation	Body weight to weaning
Organ weights[c]	Lactation	Skeletal evaluation	Surface righting
Histopathology[d]			Functional observational battery, startle response
Task 3: Crossover mating			
NA			
Task 4: F1 offspring mating and assessment			
F1c weanling	**F1c adult systemic**	**F1c adult reproductive**	**F2a, b, c pups**
Testicular descent	Mortality	Sperm analysis	Litter observations[a]
Hypospadias	Body weight, food consumption	Vaginal cytology	Anogenital distance
Nipple retention	Thyroid hormone levels[b]	Pregnancy index	
Cleft clitoris	Necropsy	Gestation	
Vaginal thread	Organ weights[c]	Lactation	
Preputial separation	Histopathology[d]		
Vaginal opening			
Vaginal cytology			
Organ weights[c]			
Necropsy			

[a]Litter observations include live and dead pups, sex ratio, and litter and individual body weights.
[b]Included T3, T4, and TSH. Results not provided in study.
[c]The following organs were weighed: liver, kidneys, spleen, thyroid/parathyroid, thymus, testes, epididymis, ventral and dorsolateral prostate, seminal vesicles with coagulating glands, ovaries, and uterus/cervix/vagina.
[d]The following organs were examined microscopically in control and high-dose animals: liver, kidneys, pituitary, spleen, thyroid/parathyroid, thymus, forestomach, testes, epididymis, and uterus/cervix/vagina.

Source: Wolfe and Pepperl (2005).

Wolfe and Pepperl (2005) did not report the results of thyroid hormone analyses or an immunological assay mentioned briefly in the methods section, noting that these results would be published separately. In F0 parental animals, there were no treatment-related effects on mortality and the incidence of clinical signs. Food consumption was slightly increased in the exposed groups during Weeks 6 and 16. F0 males exposed to 12.5 mg/kg-day had statistically significantly ($p < 0.05$) decreased total sperm per cauda (25% less than controls), statistically significantly ($p < 0.05$) decreased body weight, and a large increase in abnormal sperm (80-fold higher than controls) (see Table 4). Among F0 females, no differences were found in estrus cycle length, number of cycles, or in number of cycling females; however, the number of females with regular cycles was significantly reduced in the 2.5- and 12.5-mg/kg-day groups (see Table 4). At termination, a number of statistically significant ($p < 0.05$) organ weight changes were observed (increased liver, kidney, thyroid, and spleen weights, as well as decreased thymus, epididymis, and prostate weights); however, the study authors concluded that only the liver-weight changes were related to treatment, as histopathology findings were limited to the liver (increased incidences of hepatocellular degeneration and fatty degeneration in the high-dose group; data not shown). Absolute and relative liver weights were increased by 25–39% in F0 males and females exposed to 12.5 mg/kg-day, and absolute liver weight was increased by 9% in F0 females exposed to 2.5 mg/kg-day, as shown in Table 4.

Table 4. Selected Findings in Rats Treated with Hexachlorobenzene in a Continuous Breeding Protocol

Endpoint	Dose in mg/kg-day			
	Control	0.5	2.5	12.5
F0				
Absolute liver weight, male (g)	25.72 ± 0.702[a]	25.58 ± 0.74	25.61 ± 0.70	32.59 ± 1.02[b]
Relative liver weight, male (mg/g bw)	33.84 ± 0.61	34.05 ± 0.59	34.60 ± 0.58	46.98 ± 1.0[b]
Absolute liver weight, female (g)	13.01 ± 0.30	12.87 ± 0.25	14.24 ± 0.40[b]	17.13 ± 0.55[b]
Relative liver weight, female (mg/g bw)	35.32 ± 0.61	36.01 ± 0.60	36.80 ± 0.74	44.06 ± 1.29[b]
Total sperm per cauda	26.84 ± 1.09	27.72 ± 0.883	24.13 ± 0.989	20.02 ± 1.577[b]
Percent abnormal sperm (%)	0.1 ± 0.07	NA	NA	8.1 ± 3.61[b]
Number females with normal cycle length	16/18[c]	16/20	11/20[b]	8/19[b]
F1a and F1b Litters				
Incidence of litters with skeletal variations, F1a	6/19	8/20	7/20	17/20[d]
Incidence of litters with skeletal variations, F1b	6/18	3/18	5/19	14/20[d]
F1c Litters				
Pup survival to PND 4	0.96 ± 0.026	0.99 ± 0.008	1.00 ± 0.004	0.77 ± 0.068[b]
Average pup weight PND 4, male (g)	9.71 ± 0.476	10.86 ± 0.494	10.47 ± 0.396	7.72 ± 0.252[b]
Average pup weight PND 4, female (g)	9.34 ± 0.441	10.42 ± 0.383	9.98 ± 0.461	7.49 ± 0.294[b]
Live pups per litter, PND 4–21	8.0 ± 0.00	7.8 ± 0.17	7.0 ± 0.49[b]	6.8 ± 0.48[b]
Day of preputial separation	42.9 ± 0.41	43.2 ± 0.38	44.8 ± 0.49[b]	NA

[a]Mean ± standard error
[b]Significantly different from control at $p < 0.05$
[c]Number affected/number examined
[d]Significantly different from control at $p < 0.05$ by Fisher's exact test performed for this review.

Source: Wolfe and Pepperl (2005).

In the F1a and F1b litters, there were no effects on litter parameters, thyroid hormone levels, or the incidences of malformations (Wolfe and Pepperl, 2005). The incidence of litters with skeletal variations was statistically significantly ($p < 0.05$) increased at the high dose of 12.5 mg/kg-day; the types of variations were not reported. The only other statistically significant ($p < 0.05$) finding was increased AGD in F1a males (8% greater than controls) and F1b females (4% greater than controls) exposed to 12.5 mg/kg-day.

All of the F1c pups exposed to 12.5 mg/kg-day died by PND 9; the cause of death was not reported. Body weight was decreased significantly ($p < 0.05$) in these pups by 20–25% compared with control values. Among surviving F1c pups, a delay in preputial separation was noted in males exposed to 2.5 mg/kg-day (see Table 4). The only statistically significant ($p < 0.5$) findings in the neurotoxicity tests of surviving F1c male pups were not biologically significant: surface-righting reflex was accelerated by 1.2 days, and hindlimb grip strength was increased by 38% in males exposed to 2.5 mg/kg-day. Analysis of sperm in F1c parents did not reveal any statistically significant differences among the groups. In addition, there were no effects on vaginal cytology, pregnancy, gestation, or lactation indices. No effects on litter observations or AGD were noted in F2a, b, or c litters; as noted earlier, F2 pups were not examined for teratogenic effects or thyroid hormone levels.

The study authors concluded that hexachlorobenzene produced general toxicity (e.g., increased liver weights and increased incidences of hepatocellular degeneration and fatty changes) and developmental toxicity (e.g., pup mortality and decreased pup weights) at 12.5 mg/kg/day, and that there was no evidence of reproductive toxicity at any dose (Wolfe and Pepperl, 2005). EPA (2009) considers the 2.5-mg/kg-day dose to be a systemic and developmental LOAEL based on increased absolute liver weight in F0 females, decreased number of F0 females with normal cycle length, decreased number of live F1c pups per litter between PNDs 4 and 21, and delayed preputial separation in F1c offspring. The low dose, 0.5 mg/kg-day, is a NOAEL.

Short-term Studies Published Since ATSDR (2002)

Hadjab et al. (2004)—Hexachlorobenzene (purity not stated) in olive oil was administered by daily gavage to male Sprague-Dawley rats (12/dose) at doses of 0, 0.16, 4, or 16 mg/kg-day for 28 days to study auditory function and thyroid hormone status (Hadjab et al., 2004). Each rat had an electrode implanted in the vicinity of the left auditory nerve prior to hexachlorobenzene treatment. Cochlear sensitivity was assessed by measuring the threshold of auditory nerve compound action potential (CAPs). CAP and thyroid hormone levels (blood and to total plasma concentrations of tri-iodothyronine [T_3] and thyroxine [T_4]) were measured prior to initiation of the study and after 1, 2, 3, and 4 weeks of exposure. Cochleae were removed, dissected, and examined by light and scanning electron microscopy after the final CAP measurements and blood sample collection.

No loss of acoustic sensitivity or histological changes was noted in rats that received 0.16 mg/kg-day (Hadjab et al., 2004). Deficits in cochlear sensitivity as determined by CAP were noted at doses ≥4 mg/kg-day. At 4 mg/kg-day, the deficits were in the mid-frequency range (2–16 kHz), and there was recovery following cessation of treatment. However, at a dose of 16 mg/kg-day, deficits were observed over a broader range of frequencies (1–32 kHz), and the change was irreversible. Morphological analyses revealed no loss of cochlear hair cells (<1% of

inner hair cells affected in the highest-dose group) or changes in stereocilia at any dose. The effect of hexachlorobenzene on thyroid hormone levels is less clear. Statistically significant ($p < 0.05$) decreases in T_4 were noted after 1 week of exposure at 4 mg/kg-day (about half the control value) and after 4 weeks of exposure at the 4- and 16-mg/kg-day doses (approximately half of the control value). There was little change in T_3 levels compared with control values, with the exception of a significant increase (less than double) in the low-dose group after 1 week of exposure. The NOAEL for this study is 0.16 mg/kg-day. The LOAEL is 4 mg/kg-day for deficits in cochlear sensitivity.

Bitri et al. (2007)—Note: This study is in French with an abridged English version and an English abstract. Hexachlorobenzene (purity not stated) in olive oil was administered to male and female Mongolian gerbils (groups of 15–18/sex/dose) via gavage at doses of 0, 1.6, 4, or 16 mg/kg-day for 30 days (Bitri et al., 2007). The purpose of the study was to investigate the effects of hexachlorobenzene on the liver and thyroid. Body weight, organ weights, plasma liver enzyme concentrations (AST and ALT), and plasma thyroid hormone concentrations (T_3 and T_4) were evaluated upon terminal sacrifice after 30 days of exposure. Relative liver weights increased statistically significantly ($p < 0.01$) in the 4- and 16-mg/kg-day groups. In the highest-dose group, T_4 levels decreased statistically significantly ($p < 0.01$) in males, and T_3 levels decreased statistically significantly ($p < 0.01$) in females (see Table 5); no other statistically significant effects on thyroid hormones were noted. Plasma ALT activity was 2- to 3-fold higher than controls in high-dose males and females (see Table 5) but was not affected at lower doses. There were no significant differences in AST activity between treated and control animals. Histological changes in the liver were dose related and consisted of centrilobular congestion and cellular necrosis (≥1.6 mg/kg-day), centrilobular and periportal vein congestion, cellular necrosis, and cytoplasmic vacuolization (≥4 mg/kg-day). The aforementioned effects were more severe, with more pronounced vacuolization and the disappearance of cellular junction, at the highest-dose level (16 mg/kg-day). No quantification of incidence or severity of these effects was presented. The LOAEL for this study is 1.6 mg/kg-day (the lowest dose tested) based on hepatocellular necrosis and centrilobular congestion at this dose.

Table 5. Significant Effects on the Liver and Thyroid of Mongolian Gerbils Exposed to Hexachlorobenzene by Gavage for 30 Days

Endpoint	Dose (mg/kg-day)			
	0	1.6	4	16
Males				
ALT (U/L)	52.66 ± 8.29[a]	not reported[b]	not reported	170 ± 24.7[c]
T_4 (nmol/l)	40.59 ± 1.08	not reported	not reported	21.95 ± 7.46[c]
Females				
ALT (U/L)	56 ± 5	not reported	not reported	120 ± 12.47[c]
T_3 (nmol/L)	3.96 ± 0.48	not reported	not reported	1.42 ± 0.11[c]

[a]Mean ± SEM, n = 15–18/dose but precise values were not specified for each endpoint.
[b]Only control and significant values were reported in the text.
[c]$p < 0.01$.

Source: Bitri et al. (2007).

Bitri et al. (2008)—Note: This study is in French with an abridged English version and an English abstract. Hexachlorobenzene (purity not stated) mixed with olive oil was administered daily by gavage to male Mongolian gerbils (15–18 per dose group) at doses of 0, 1.6, 4, or 16 mg/kg-day for 30 days (Bitri et al., 2008). Plasma testosterone concentrations were measured (details of timing were not provided in the abridged English version), and testes and seminal vesicles were evaluated histologically. Significant results are shown in Table 6. Plasma testosterone concentrations were statistically significantly decreased ($p < 0.01$) at doses of 4 mg/kg-day (0.48 ± 0.08 ng/ml) and 16 mg/kg-day (0.54 ± 0.07 ng/ml) compared to controls (1.08 ± 0.1 ng/ml). Hexachlorobenzene exposure had no effect on the diameter of the seminiferous tubules, but a decrease in spermatozoid content was observed in parallel with decreased testicular "spermatic activity" in the testes (this measure was not defined) at all doses; relative seminal vesicle weight was unaffected by treatment. Relative testes weight (relative to body weight) was significantly decreased at all doses (see Table 6). The LOAEL for this study is 1.6 mg/kg-day (lowest dose tested) for decreased testicular spermatozoid content and relative testes weight.

Table 6. Significant Effects on the Testes of Mongolian Gerbils Exposed to Hexachlorobenzene by Gavage for 30 Days

Endpoint	Dose (mg/kg-day)			
	0	1.6	4	16
Relative testes weight (% body weight)	1.6 ± 0.03[a]	1.25 ± 0.07[b]	1.38 ± 0.09[c]	1.41 ± 0.07[c]
"Spermatic activity" in testes (%, not further defined)	88 ± 4.89	82 ± 5.83[b]	80 ± 5.77[b]	60 ± 3.16[b]
Plasma testosterone (ng/mL)	1.08 ± 0.01	not reported	0.48 ± 0.08[b]	0.54± 0.07[b]

[a]Mean ± SEM, n = 15–18/dose but precise values were not specified for each endpoint.
[b]$p < 0.01$
[c]$p < 0.05$

Source: Bitri et al. (2008).

Chiappini et al. (2009)—Hexachlorobenzene (>99% purity) was administered as a suspension in water containing Tween 20 (0.5/100 ml) by gavage to Wistar rats (3 females/dose) at doses of 0, 0.1, 1, 10, 100, or 500 mg/kg-day, 5 days/week, for 30 days (Chiappini et al., 2009). The objective of the study was to assess the possible disruptive effects of hexachlorobenzene on thyroid growth regulation, apoptosis, and cell proliferation in thyroid tissue. On the day following the final dose, thyroid hormones in the plasma were measured by a commercial chemoluminescence kit. Histomorphology was assessed in thyroid glands; a subset of 200 follicles taken from the glands of three rats per dose was selected for parametric measurement of follicle morphology. Follicular cell proliferation was measured in animals given an intraperitoneal injection of bromodeoxyuridine (BrdU) 30 minutes prior to terminal sacrifice; thyroid sections were incubated with mouse antiBrdU, and then stained. The number of labeled cells with BrdU compared to the total number of cells in the selected area was used as the measure of proliferation. Apoptotic nuclei were identified in thyroid sections by the detection of DNA breaks with the Terminal Transferase dUTP Nick End Labeling (TUNEL) technique. Thyroid growth factor β-1 (TGF-β-1) mRNA expression was assessed by reverse transcriptase-polymerase-chain-reaction analysis. Protein fractions, including cytochrome C, active caspase 8 (results of procaspase-8 cleavage), and active caspase 9 (result of procaspase-9

cleavage), in thyroid follicular cells were assessed by Western blotting with appropriate antibodies.

No effects were observed in rats exposed to 0.1 mg/kg-day (Chiappini et al., 2009). At doses ≥1 mg/kg-day, dose-related increases in cytochrome C, active caspase-9, increases in expression of TGF-β-1, and significant ($p < 0.01$) increases in apoptosis were observed. No changes in active caspase-8 were observed at any dose. However, there was no significant effect on thyroid-follicular cell proliferation, as evaluated by BrdU incorporation into DNA, and no effects on T_3 or TSH levels. At 500 mg/kg-day, T_4 was significantly decreased relative to controls (see Table 7), and significant morphological changes (increase in the colloid area with a decrease in epithelial height) occurred. Thyroid weight relative to body weight was not affected at any dose including the highest dose. In addition, no other effects were seen at any of the lower doses. The study authors concluded that hexachlorobenzene does not disrupt thyroid hormone economy, cell proliferation, or cell morphology at doses <500 mg/kg-day but is capable of initiating induction of TGF-β-1 expression and the cascade of effects on cytochrome C and procaspase-9 that result in the observed apoptotic response[3] at doses of ≥1 mg/kg-day. The NOAEL for this study is 100 mg/kg-day, and the LOAEL is 500 mg/kg-day on the basis of significant decreased circulating T_4 and morphological changes in the thyroid.

Table 7. Significant Effects on the Thyroid of Female Wistar Rats Exposed to Hexachlorobenzene by Gavage for 30 Days

		Dose (mg/kg-bw)				
Endpoint	**0**	**0.1**	**1**	**10**	**100**	**500**
T_4 (µg/dL)	3.67 ± 0.52[a]	NA	3.55 ± 0.43	3.88 ± 0.36	3.32 ± 0.29	2.21± 0.19[b]

[a]Mean ± SEM.
[b]Significantly different from control rats ($p < 0.05$).

Source: Chiappini et al. (2009).

Inhalation Exposure

Studies relevant to the derivation of provisional chronic or subchronic inhalation RfC values were not located.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR HEXACHLOROBENZENE

SUBCHRONIC p-RfD

There are a large number of short-term and subchronic oral studies of hexachlorobenzene. Given the size of the toxicological database, this review focused on those studies most relevant to derivation of a subchronic p-RfD. The studies considered pertinent to subchronic p-RfD derivation included (1) the principal study (Arnold et al., 1985) used to derive the chronic RfD on IRIS (U.S. EPA, 2009) and the chronic-duration oral MRL (ATSDR, 2002), as this study included a subchronic-duration exposure component (F0 generation); (2) the key

[3]Cytochrome C and capsase-9 are mitochondrial upstream regulatory elements in proposed apoptotic pathways.

studies used by ATSDR (2002) to derive the intermediate-duration oral MRL; and (3) any short-term, subchronic, developmental, and reproductive toxicity studies published since the ATSDR (2002) toxicological profile. These studies are summarized in Table 8. The principal study that has been chosen for the determination of the subchronic RfD is Bourque et al. (1995), which provided the lowest LOAEL (0.01 mg/kg-day) representing the most sensitive effect in the subchronic database (see Table 8). The critical effect is degenerative changes in primary ovarian follicles of monkeys; the selection of the POD is supported by other studies that reported degenerative changes in ovarian follicles and surface epithelium in monkeys given hexachlorobenzene for 90 days with an overall LOAEL of 0.1 mg/kg-day (Jarrell et al., 1993; Foster et al., 1992, 1995; Babineau et al., 1991; Sims et al., 1991). Furthermore, these studies provide evidence that hexachlorobenzene is toxic to the mammalian ovary and may interfere with mechanisms regulating ovarian steroidogenesis (ATSDR, 2002; Foster et al., 1992). The LOAEL for the database is 0.01 mg/kg-day for degenerative changes in primary ovarian follicles of female Cynomolgus monkeys exposed to hexachlorobenzene for 13 weeks (Bourque et al., 1995). Bourque et al. (1995) did not provide quantitative data that could be used in benchmark dose (BMDL) modeling to identify a point of departure (POD). Therefore, the POD selected for the derivation of a subchronic p-RfD is the LOAEL of 0.01 mg/kg-day from Bourque et al. (1995).

A **subchronic p-RfD** was derived as follows:

Subchronic p-RfD = LOAEL ÷ UF
= 0.01 mg/kg-day ÷ 1000
= **0.00001 or 1×10^{-5} mg/kg-day**

The composite UF of 300 is composed of the following:

- UF_A: A factor of 10 is applied for animal-to-human extrapolation to account for toxicokinetic and dynamic differences between monkey and humans. There are no data to determine whether humans are more sensitive than monkeys to ovarian degeneration.
- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating a susceptible human response are insufficient.
- UF_D: The database for oral exposure to hexachlorobenzene is extensive and includes both developmental and multigeneration reproductive toxicity studies, as reviewed by ATSDR (2002). A factor of 1 is applied for database inadequacies because all requirements for database completeness are satisfied and additional sources of data seem unlikely to result in a lower POD.
- UF_L: A factor of 10 is applied for extrapolation from a LOAEL to a NOAEL.

Table 8. Summary of Oral Noncancer Dose-response Information Pertinent to Derivation of Subchronic p-RfD

Species (*n*/sex/group)	Exposure	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Duration-adjusted[a] NOAEL (mg/kg-d)	Duration-adjusted[a] LOAEL (mg/kg-d)	Responses at the LOAEL	Comments	Reference
Principal Study for Chronic RfD (U.S. EPA, 2009) and Chronic-Duration Oral MRL (ATSDR, 2002)								
Rat, Sprague-Dawley (64–66/sex/group)	0, 0.32, 1.6, 8.0, or 40.0 ppm in the diet (0, 0.03, 0.14, 0.69, and 3.4 mg/kg-d for males and 0, 0.03, 0.16, 0.78, and 3.9 mg/kg-d for females) for 90 d premating, and possibly during gestation and lactation for one generation	0.69 0.14	3.4 0.69	0.69 0.14	3.4 0.69	Reproductive toxicity: Decreased viability of offspring between birth and PND 4 Parental toxicity: increased organ weights (liver, heart, and brain weight and liver:body-weight ratio in males)	Data for organ weights were not shown. F1 generation was exposed chronically; F1 results were not considered for subchronic p-RfD derivation	Arnold et al. (1985)
Critical Studies for ATSDR Intermediate-Duration Oral MRL (ATSDR, 2002)								
Monkey, Cynomolgus (4 F/group)	0, 0.1, 1, or 10 mg/kg-d in gelatin capsules for 90 d	ND	0.1	ND	0.1	Degenerative changes in ovarian follicles and surface epithelium		Jarrell et al. (1993); Foster et al. (1992); Babineau et al. (1991); Sims et al. (1991)
Monkey, Cynomolgus (4 F/group)	0, 0.01, 0.1, 1.0, or 10 mg/kg-d in gelatin capsules for 13 wks	ND	0.01	ND	0.01	Degenerative changes in primary ovarian follicles		Bourque et al. (1995)
Monkey, Cynomolgus (4 F/group)	0, 0.1, 1.0, or 10 mg/kg-d in capsules for 90 d	1	10	1	10	Increased mean menstrual cycle length was accompanied by reduced serum estradiol during ovulation		Foster et al. (1995)

Table 8. Summary of Oral Noncancer Dose-response Information Pertinent to Derivation of Subchronic p-RfD

Species (*n*/sex/group)	Exposure	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Duration-adjusted[a] NOAEL (mg/kg-d)	Duration-adjusted[a] LOAEL (mg/kg-d)	Responses at the LOAEL	Comments	Reference
Subchronic and Short-Term Developmental, or Reproductive Toxicity Studies Published since ATSDR (2002)								
Rat, Sprague-Dawley (9–10 F/group)	0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, or 25 mg/kg-d by gavage, 5 d/wk, for 13 wks	0.3	1.0	0.2	0.71	Pulmonary interstitial fibrosis and histolytic infiltration; maxillary incisor degeneration; decreased free T_4	The NTP has not yet published a full report of this study	Long et al. (2004), Johnson et al. (2005, abstract only)
Rat, Sprague-Dawley (20/group)	0, 0.5, 2.5, or 12.5 mg/kg-d by daily gavage for two generations in a continuous breeding protocol	0.5	2.5 (systemic and develop-mental)	0.5	2.5 (systemic and develop-mental)	Increased absolute liver weight in F0 females; decreased number of F0 females with normal cycle length; decreased number of live F1c pups per litter (PND 4–21), and delayed preputial separation in F1c litters.		Wolfe and Pepperl (2005)
Rat, Sprague-Dawley (12 M/group)	0, 0.16, 4 or 16 mg/kg-d by gavage daily for 28 d	0.16	4	0.16	4	Deficits in cochlear sensitivity without cochlear hair cell loss or ciliary changes	Study of auditory function	Hadjab et al. (2004)
Gerbil, Mongolian (15–18/sex/dose)	0, 1.6, 4, or 16 mg/kg-d by gavage for 30 d	ND	1.6	ND	1.6	Hepatocellular necrosis and centrilobular congestion	Study published in French with abridged English version and not translated for this review	Bitri et al. (2007)
Gerbil, Mongolian (15–18 M/dose) gavage	0, 1.6, 4, or 16 mg/kg-d, by gavage for 30 d	ND	1.6	ND	1.6	Decreased spermatozoid content and relative weight of testes; also sperm activity (not further described)	Study published in French with abridged English version and not translated for this review	Bitri et al. (2008)

Table 8. Summary of Oral Noncancer Dose-response Information Pertinent to Derivation of Subchronic p-RfD

Species (*n*/sex/group)	Exposure	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Duration-adjusted[a] NOAEL (mg/kg-d)	Duration-adjusted[a] LOAEL (mg/kg-d)	Responses at the LOAEL	Comments	Reference
Rat, Wistar (3 F/dose)	0, 0.1, 1, 10, 100, or 500 mg/kg-d by gavage, 5 d/wk, for 30 d	100	500	71	357	Changes in thyroid cell morphology and thyroid hormone levels.	Investigation limited to thyroid effects	Chiappini et al. (2009)

[a]Adjusted for continuous exposure as follows: $NOAEL_{adj}$ = NOAEL × Exposure Days/7 days.

Confidence in the principal study is medium. The study was conducted for a 90-day time period and conducted a thorough evaluation of the target organ; in addition, the critical effect is supported by other monkey studies: Jarrell et al. (1993), Foster et al. (1992, 1995), Babineau et al. (1991), and Sims et al. (1991). However, the number of monkeys per group (n = 4) tested by Bourque et al. (1995) was small, and a NOAEL for the critical effect was not defined. Confidence in the database is high. There are numerous subchronic toxicity studies in multiple species, reproductive, and developmental toxicity studies in multiple species, and short-term, repeated-dose toxicity studies (see ATSDR, 2002). Medium confidence in the p-RfD follows.

CHRONIC p-RfD

IRIS (U.S. EPA, 2009) contains a chronic p-RfD for hexachlorobenzene of 8×10^{-4} mg/kg-day, based on a NOAEL of 0.08 mg/kg-day (estimated from a dietary concentration of 1.6 ppm and a food factor of 5%) for liver effects in F1 rats exposed chronically in the study by Arnold et al. (1985) and a UF of 100. The subchronic p-RfD is lower than the chronic IRIS RfD due to the availability of newer data.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR HEXACHLOROBENZENE

The existing database for hexachlorobenzene does not include studies that can be used to derive inhalation p-RfC values for hexachlorobenzene.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR HEXACHLOROBENZENE

A provisional carcinogenicity assessment was not prepared for hexachlorobenzene because IRIS (U.S. EPA, 2009) includes a cancer assessment for this compound.

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