Hexachloroethane (HCE); CASRN: 67-72-1)

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR: Hexachloroethane (HCE)

File First On-Line 03/31/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	09/23/2011
Inhalation RfC (I.B.)	yes	09/23/2011
Carcinogenicity Assessment (II.)	yes	09/23/2011

I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Substance Name — Hexachloroethane (HCE)
CASRN — 67-72-1
Last Revised — 09/23/2011

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents for an elaboration of these concepts. Because RfD values can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is

essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

A previous oral RfD of 1×10^{-3} mg/kg-day for hexachloroethane was posted on the IRIS database in 1987.

I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure	UF	Chronic RfD
Atrophy and degeneration of renal tubules **Male F344 rats** **16-week subchronic dietary exposure study** **Gorzinski et al. (1985)**	$BMDL_{10}$ = 0.728 mg/kg-day	1000	7×10^{-4} mg/kg-day

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Gorzinski et al. (1985) fed 1, 15, or 62 mg/kg-day HCE (purity 99.4%) to F344 rats (10 rats/sex/dose) for 16 weeks. At the high dose, male rats displayed statistically significant increases in absolute and relative kidney weights and gross pathological alterations. Male rats of the 62 mg/kg-day group exhibited statistically significant increases in absolute and relative liver weights; histopathology revealed a slight swelling of the hepatocytes in the 15 and 62 mg/kg-day dose groups. Female rats exhibited a statistically significant increase in relative liver weight at the high dose, although there was no evidence of hepatotoxicity in the histopathological examination. Male rats displayed slight hypertrophy and/or dilation of proximal convoluted tubules of the kidneys at incidences of 0/10, 1/10, 7/10, and 10/10 for the 0, 1, 15, and 62 mg/kg-day dose groups, respectively. The increased incidence of slight hypertrophy and/or dilation of proximal convoluted tubules was statistically significant in males at the 15 and 62 mg/kg-day doses. Male rats displayed atrophy and degeneration of renal tubules at incidences of 1/10, 2/10, 7/10, and 10/10 for the 0, 1, 15, and 62 mg/kg-day dose groups, respectively. The increased incidence of atrophy and degeneration of renal tubules was statistically significant in males at the 15 and 62 mg/kg-day doses. Female rats did

not display hypertrophy and/or dilation of proximal convoluted tubules of the kidneys, but did exhibit atrophy and degeneration of proximal tubules (1/10, 1/10, 2/10, and 6/10 at the 0, 1, 15, and 62 mg/kg-day doses, respectively). The increased incidence of atrophy and degeneration of proximal tubules was statistically significant in females at the 62 mg/kg-day dose.

The authors concluded the no-observed-effect level (NOAEL) for both male and female rats was 1 mg/kg-day. For male rats, EPA considered 1 mg/kg-day as NOAEL and 15 mg/kg-day as the lowest-observed-adverse-effect level (LOAEL), based on renal tubule toxicity. For female rats, EPA considered the NOAEL as 15 mg/kg-day and the LOAEL as 62 mg/kg-day, based on renal tubule toxicity

Methods of Analysis. Atrophy and degeneration of renal tubules in male F344 rats as described by Gorzinski et al. (1985) was selected as the critical effect for the derivation of the RfD. All of the dichotomous dose-response models available in the EPA benchmark dose software (BMDS), version 2.0, were fit to the incidence data for kidney effects in male rats (Gorzinski et al., 1985). Details of the BMD dose response modeling reported in Appendix B of the Toxicological Review of Hexachloroethane (Table B-1). A benchmark response (BMR) of 10% extra risk was considered appropriate for derivation under the assumption that it represents a minimally biologically significant response level.

The most sensitive effect observed in male rats exposed to HCE was slight hypertrophy and/or dilation of proximal convoluted renal tubules (Gorzinski et al., 1985); however, the candidate POD for slight hypertrophy and/or dilation of proximal convoluted renal tubules (i.e., 0.710 mg/kg-day) is nearly identical to the candidate POD for atrophy and degeneration of renal tubules (i.e., 0.728 mg/kg-day). As tubular nephropathy in the chronic studies (NTP, 1989; NCI, 1978) was characterized as atrophy and degeneration of renal tubules, this endpoint has been consistently observed following HCE exposure in several studies. Therefore, atrophy and degeneration of renal tubules was selected as the candidate critical effect for male rats exposed to HCE.

The gamma, multistage 1°, logistic, probit, quantal-linear, and Weibull models in BMDS (version 2.0) provided adequate fits to the incidence data for atrophy and degeneration of renal tubules in male rats from the (Gorzinski et al., 1985) 16-week study (Table B-1 of the Toxicological Review of Hexachloroethane), as assessed by a X^2 goodness-of-fit p-values. BMD_{10} and $BMDL_{10}$ estimates from these models were within a factor of three of each other, suggesting no appreciable model dependence. The models with the lowest Akaike's information criterion (AIC; a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint) values were for the gamma, multistage 1°, and quantal-linear models; therefore, the model with the lowest $BMDL_{10}$ was selected. These

models had identical BMD_{10} and $BMDL_{10}$ values. Therefore, the $BMDL_{10}$ of 0.728 mg/kg day associated with a 10% extra risk for nephropathy in male rats was selected as the POD for these data and serves as the basis for the derivation of the oral RfD for HCE. This endpoint is supported by additional kidney effects associated with oral exposure to HCE and supports the weight of evidence for HCE-associated nephrotoxicity.

I.A.3. UNCERTAINTY FACTORS

UF = 1000

An interspecies uncertainty factor, UF_A, of 10 was applied to account for uncertainty in extrapolating from laboratory animals to humans in the absence of information to characterize the toxicokinetic or toxicodynamic differences between rats and humans after oral HCE exposure. Although the toxicokinetics have been minimally evaluated in animals, the toxicokinetics of HCE have not been sufficiently characterized in either rats or humans to identify the active compound or determine dose metrics.

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to oral HCE exposure in the human population.

A subchronic-to-chronic UF (UF_S) of 3 was applied. The study selected as the principal study was the 16-week study by Gorzinski, et al. (1985), a study duration that is minimally past the standard subchronic (90-day) study and falls well short of a standard lifetime study (i.e., two year chronic bioassay). Some chronic data (NTP, 1989; Gorzinski et al., 1985; NCI, 1978) were available to inform the nature and extent of effects that would be observed with a longer duration of exposure to HCE. The chronic data identified the kidney is the target organ of HCE toxicity, consistent with the findings from the Gorzinski et al. (1985) study. Increases in severity of tubular nephropathy in the NTP (1989) chronic study was reported at similar doses as atrophy and degeneration of renal tubules in the Gorzinski et al. (1985) subchronic study, suggesting consistency in dose response relationships between chronic and subchronic studies. In addition, data from the NCI (1978) chronic study suggested that an increase in duration of HCE exposure may not increase the incidence of nephropathy. However, the lowest dose tested in the chronic exposure studies (NTP, 1989; NCI, 1978) represented a LOAEL, limiting the ability of these studies to inform the impact of increased exposure duration on renal effects observed at the lowest dose in the subchronic study (Gorzinski et al., 1985). For these reasons, a UF_S of 3 was used to account for extrapolation from subchronic to chronic exposure duration.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in the incidence of renal tubule atrophy and degeneration was selected under an assumption that it represents a minimal biologically significant change

A database uncertainty factor, UF_D, of 3 was applied to account for database deficiencies due to the lack of a multigenerational reproductive study. The database includes studies in laboratory animals, including chronic and subchronic dietary exposure studies and two oral developmental toxicity studies.

I.A.4. ADDITIONAL STUDIES/COMMENTS

The predominant noncancer effect of acute, short-term, subchronic, and chronic oral exposure to hexachloroethane is renal toxicity. The acute and short-term study data were not considered in the selection of the principal study for the derivation of the RfD because the database contained dose-response data from studies of subchronic and chronic durations. In addition to the Gorzinski et al. (1985) study, two chronic studies in rats (NTP, 1989; NCI, 1978), a chronic study in mice (NCI, 1978), and a subchronic study in rats (NTP, 1989) support the selection of the kidney as the target organ, and atrophy and degeneration of renal tubules as the critical effect of hexachloroethane exposure.

In the NTP (1989) chronic study, hexachloroethane was administered via gavage at doses of 7 and 14 mg/kg-day in male F344 rats and 57 and 114 mg/kg-day in female F344 rats for 103 weeks. Nephropathy (characterized by tubular cell degeneration and regeneration, tubular dilatation and atrophy, glomerulosclerosis, interstitial fibrosis, and chronic inflammation) was observed in hexachloroethane-treated rats of both sexes. Nephropathy was also reported in control rats of both sexes. Although a high incidence of nephropathy was observed in control rats, the study authors reported that the incidence of more severe nephropathy increased in dosed rats relative to controls (NTP, 1989). EPA considered the increase in severity of nephropathy in male rats by analyzing the incidences of greater than mild nephropathy. EPA determined that the increased incidences of moderate or marked nephropathy in males were statistically significant at the 14 mg/kg-day dose (see Table 5-1 of the Toxicological Review of Hexachloroethane). EPA considered the increased severity of nephropathy in female rats by analyzing the incidences of nephropathy that were greater than minimal nephropathy. EPA determined that the increased incidences of mild to moderate nephropathy were statistically significant in females at the 57 and 114 mg/kg-day doses (see Table 5-1 of the Toxicological Review of Hexachloroethane). Linear mineralization of the renal papillae and hyperplasia of the renal pelvic epithelium were increased in a dose-dependent, statistically significant manner in the treated male rats. EPA determined that the increased incidences of linear mineralization of the renal papillae and hyperplasia of the renal pelvic epithelium were statistically

significant in males at the 7 and 14 mg/kg-day doses (see Table 5-1 of the Toxicological Review of Hexachloroethane). The increased severity of nephropathy and dose-dependent increases in the incidence of mineralization of the renal papillae and hyperplasia of renal pelvic transitional epithelium in male rats suggested that hexachloroethane exposure exacerbated the nephropathy observed in the NTP (1989) study. The NTP (1989)chronic study did not identify NOAELs for male or female rats, as kidney effects were observed at the lowest doses tested. EPA considered the male rat LOAEL as 7 mg/kg-day based on increased incidence in moderate or marked tubular nephropathy (characterized by degeneration, necrosis, and regenerative epithelial cells), hyperplasia of the pelvic transitional epithelium, and linear mineralization of the renal papillae in the NTP (1989) study. EPA considered the female rat LOAEL as 57 mg/kg-day, based on dose-related increases in incidence and severity of nephropathy in the NTP (1989) study.

In the NCI (1978) chronic rat study, hexachloroethane was administered via gavage to groups of 50 male and 50 female Osborne-Mendel rats for 5 days/week, (cyclically for 66 of the 78 weeks of exposure), followed by an observation period of 33–34 weeks (total study duration of 112 weeks). The TWA doses of hexachloroethane were 113 and 227 mg/kg-day. Tubular nephropathy was observed in all groups of treated animals, but was not observed in either untreated or vehicle controls. Statistically significant increases in incidence of tubular nephropathy were observed at 113 and 227 mg/kg-day hexachloroethane in both male and female rats (see Table 5-1 of the Toxicological Review of Hexachloroethane). The NCI (1978) study did not identify a NOAEL for tubular nephropathy in rats. EPA considered the LOAEL as 113 mg/kg-day, based on a dose-related increase in incidence of nephropathy in both male and female rats.

In the NCI (1978) chronic mouse study, hexachloroethane was administered via corn oil gavage to groups of 50 male and 50 female B6C3F_1 mice for 5 days/week for 78 weeks followed by an observation period of 12–13 weeks (total study duration of 91 weeks). Starting in week 9, the hexachloroethane doses were increased, though no explanation for the increase was provided. The TWA doses of hexachloroethane were 360 and 722 mg/kg-day. Because of low survival rates in the vehicle and untreated male control groups, NCI (1978) compared tumor incidences in the dosed males and females to the pooled vehicle control data derived from concurrently run bioassays for several other chemicals. NCI (1978) reported chronic kidney inflammation (i.e., tubular nephropathy characterized by degeneration of the convoluted tubule epithelium at the junction of the cortex and medulla and hyaline casts) in male and female B6C3F_1 mice administered 360 and 722 mg/kg-day hexachloroethane. EPA considered the LOAEL for this study as 360 mg/kg-day based on tubular nephropathy, while a NOAEL could not be established from these data.

In the NTP (1989) subchronic study, hexachloroethane was administered via gavage to groups of 10 male and 10 female F344 rats at TWA doses of 0, 34, 67, 134, 268, and 536 mg/kg-day for 13 weeks. Kidney effects (i.e., hyaline droplet formation, renal tubular regeneration, and renal tubular casts) were observed in male rats from all hexachloroethane exposure groups, though incidence data were only provided for the 34 mg/kg-day dose group. NTP (1989) reported that the severity of kidney effects in male rats increased with dose, but no data on severity were presented. No kidney effects were reported in female F344 rats exposed to hexachloroethane. Liver effects were observed in male and female rats at higher doses of hexachloroethane and EPA determined that statistically significant increases in hepatocellular necrosis were observed in female rats exposed to 268 or 536 mg/kg-day hexachloroethane (see Table 5-1 of the Toxicological Review of Hexachloroethane).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF).

I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study — High
Database — Low to Medium
RfD — Low to Medium

Overall confidence in the RfD is low to medium. Confidence in the principal study, Gorzinski et al. (1985), is high. The 16-week study was a well-conducted study that used three dose groups plus a control. NTP (1989) also conducted 16-day, 13-week, and 103-week studies that supported the results observed in the 16-week study. Application of BMD modeling provided a POD upon which to base the derivation of the RfD. The critical effect, on which the RfD is based, was well-supported by other oral short-term, subchronic, and chronic studies. Confidence in the database is low to medium because the database included acute, short-term, subchronic, and chronic toxicity studies and developmental/teratogenic toxicity studies in rats and chronic carcinogenicity bioassays in rats and mice. The database lacks a multigenerational reproductive study and studies in other species.

For more detail on Dose-Response Assessments, exit to the toxicological review, Section 5 (PDF).

I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document – *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

Agency Completion Date – 09/23/2011

I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Substance Name — Hexachloroethane (HCE)
CASRN — 67-72-1
Section I.B. Last Revised — 09/23/2011

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. A summary of the evaluation of potential human carcinogenicity of hexachloroethane is contained in Section II of this file.

An inhalation assessment for hexachloroethane was not previously available on IRIS.

I.B.1. CHRONIC INHALATION RfC SUMMARY

Critical Effect	Point of Departure	UF	Chronic RfC
Neurotoxicity (tremors and ruffled pelt) **Male and female Sprague-Dawley rats** **6-week subchronic inhalation study** **Weeks et al. (1979)**	$NOAEL_{[HEC]}$: 83 mg/m^3	3000	3×10^{-2} mg/m^3

*Conversion Factors and Assumptions – The POD from this study was adjusted for continuous exposure (24 hours/day, 7 days/week). $POD_{[ADJ]}$ = (465 mg/m^3) × (6/24 hours) × (5/7 days) = 83.0 mg/m^3. Consequently, for dosimetric purposes, the human equivalent concentration (HEC) for HCE was calculated by applying the appropriate dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases) to the duration-adjusted exposure level ($POD_{[ADJ]}$), in accordance with the U.S. EPA RfC methodology (1994a). The DAF for a Category 3 gas is based on the regional gas dose ratio (RGDR), where the RGDR is the ratio of the animal blood:gas partition coefficient $(H_{b/g})_A$ and the human blood:gas partition coefficient $(H_{b/g})_H$. The animal and human blood:gas partition coefficients for HCE are not known. In accordance with the RfC Methodology (U.S. EPA, 1994a) when the partition coefficients are unknown or $(H_{b/g})_A$ is greater than $(H_{b/g})_H$, a RGDR of 1 is used. The partition coefficients were unknown for HCE; resulting in a $POD_{[HEC]}$ of 83.0 mg/m^3.

I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

Weeks et al. (1979) exposed male, non-pregnant female, Sprague-Dawley rats (21-25/sex/concentration) to control air, 15, 48, or 260 ppm hexachloroethane (145, 465, and 2,517 mg/m^3, respectively; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks, and pregnant female rats exposed to the same concentrations for 11 days of gestation. Postexposure observations were carried out for 12 weeks. An oxygen consumption test was also conducted. The authors reported that in the 2,517 mg/m^3 group, body weight gain of male rats, but not the non-pregnant female rats, was reduced beginning in the third week of exposure (although quantitative information was not reported). All rats in the 2,517 mg/m^3 group exhibited tremors, ruffled pelt, and red exudates around the eyes following the fourth week of exposure. The authors reported that in the male rats, relative kidney, spleen, and testes weights were significantly increased; but in the non-pregnant female rats, only relative liver weights were significantly increased (although quantitative information was not reported).

One male and one non-pregnant female rat in the 2,517 mg/m^3 exposure group died during the fourth week of exposure, but the authors did not report a cause of death. During the postexposure observation period, treatment-related effects disappeared. No gross changes were evident at necropsy after the 12 week postexposure observation period; however, male and non-pregnant female rats of the 2,517 mg/m^{3}group (sacrificed immediately after the 6 week inhalation exposure) had a higher incidence and severity of mycoplasma-related lesions in nasal turbinates, trachea, and lung, compared with controls. The authors concluded that these lesions were related to potentiation of an endemic mycoplasma infection rather than a direct effect of hexachloroethane exposure. However, no data were presented demonstrating the presence of mycoplasma in the lung. There were no histopathological differences observed between control and exposed rats sacrificed 12 weeks postexposure. No treatment-related effects were observed in the rats exposed to 145 and 465 mg/m^3 hexachloroethane.

In the oxygen consumption test, male rats (5/concentration) were tested prior to and following exposure to 145, 465, or 2,517 mg/m^3 hexachloroethane for 15 minutes, 3 days/week for the duration of the study (6 weeks). The 2,517 mg/m^3 rats exhibited significantly decreased mean rates of consumption prior to (15%) and after (13%) exposure to hexachloroethane. The authors suggested that this decrease in oxygen consumption, while nonspecific, is indicative of an alteration in basal metabolic rate. No histopathological effects were observed at this concentration. EPA considered 465 mg/m^3 the NOAEL and 2,517 mg/m^3 the LOAEL, based on reduced body weight gain, and increased organ weights.

Weeks et al. (1979) also exposed male Sprague-Dawley rats (15/concentration) to 15, 48, or 260 ppm hexachloroethane (145, 465, or 2,517 mg/m^3) for 6 hours/day, 5 days/week for 6 weeks and examined them for behavioral changes related to learned and unlearned responses (described in detail in Section 4.4.3.2 of the Toxicological Review of Hexachloroethane). Similar to the other treated rats, body weight gain was reduced. Final mean body weight gain in male rats was reduced 2, 5, and 10% (statistically significant) in the 145, 465, and 2,517 mg/m^3 dose groups, respectively, compared with controls. Additionally, relative lung, liver, kidney, and testes weights were increased (quantitative information not reported) compared with controls.

Weeks et al. (1979) also exposed 16 male Beagle dogs (four dogs per concentration: control air [0], 15, 48, or 260 ppm hexachloroethane [145, 465, and 2,517 mg/m^3, respectively]; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observations were carried out for 12 weeks. Blood samples were evaluated for blood chemistry parameters. In addition, the dogs underwent pulmonary function tests prior to and following exposure. One dog died within 5 hours of exposure to 2,517 mg/m^3. The remaining animals in the 2,517 mg/m^3 group exhibited signs of neurotoxicity consisting of tremors, ataxia, hypersalivation, head bobbing, and facial fasciculations. No blood parameters were significantly affected and no exposure-

related histopathological lesions were observed following necropsy on dogs sacrificed 12 weeks postexposure. Dogs evaluated for pulmonary functions while anesthetized did not display any significant effects. The hexachloroethane-exposed dogs did not display any treatment-related toxicity at 12 weeks postexposure. EPA considered 465 mg/m^3 the NOAEL and 2,517 mg/m^3 the LOAEL, based on neurotoxic effects.

Weeks et al. (1979) also exposed male Hartley guinea pigs (10/concentration: control air [0], 15, 48, or 260 ppm hexachloroethane [145, 465, and 2,517 mg/m^3, respectively]; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observations were carried out for 12 weeks. Guinea pigs were also evaluated for sensitization potential following inhalation exposure to hexachloroethane. Two guinea pigs died during each of the fourth and fifth weeks, resulting in four total deaths. Guinea pigs of the 2,517 mg/m^3 group displayed reductions in body weight beginning at the second week of exposure and significantly increased liver to body weight ratios (quantitative information was not reported). No treatment-related effects were observed in the other exposure groups. EPA considered the NOAEL as 465 mg/m^3 and the LOAEL as 2,517 mg/m^3, based on decreased body weight and significantly increased relative liver weight.

Weeks et al. (1979) also exposed male and female quail (*C. japonica*, 20/concentration:control air [0], 15, 48, or 260 ppm hexachloroethane [145, 465, and 2,517 mg/m^3, respectively]; purity 99.8%) for 6 hours/day, 5 days/week for 6 weeks. Postexposure observations were carried out for 12 weeks. The only observed effect was excess mucus in nasal turbinates in 2/10 quail in the 2,517 mg/m^3 group after 6 weeks. The authors considered the excess mucus to be transient based on the lack of any inflammation or histopathological effects. Although the study authors considered the excess mucus to be a transient effect, EPA noted that the lack of inflammation and histopathological effects does not preclude the presence of more sensitive indicators of immune response (e.g., antibodies or other immune signaling chemicals) unable to be detected with methods available to the study authors. EPA considered 2,517 mg/m^3 (highest exposure concentration) as the NOAEL, while the LOAEL could not be established from this study.

The subchronic inhalation study by Weeks et al. (1979), as the only repeated exposure study available, was selected as the principal study for the derivation of the RfC. The Weeks et al. (1979) study was a well-conducted subchronic bioassay which used three concentrations and incorporated a variety of endpoints (e.g., toxicological, teratogenic, neurological, pulmonary) across a range of species (see Table 5-4 of the Toxicological Review of Hexachloroethane). The authors evaluated portal of entry effects by gross examination of lungs, trachea, and nasal turbinates following necroscopy on animals that died during the study or were sacrificed at 12 weeks postexposure. In addition, Weeks et al. (1979) evaluated upper respiratory effects by examining histological sections of the nasal turbinates and evaluated upper respiratory inflammation by the presence of polymorphonuclear leukocytes in close association with

excess mucus within the lumens of the nasal passages. The primary limitation of Weeks et al. (1979) was the minimal amount of quantitative information provided, characterizing the reported effects. Several experiments only utilized one sex, and additional exposure concentration(s) between the mid- and high concentrations would have allowed for better characterization of the exposure-response curve. However, this study identified neurotoxicity, statistically significant decreases in body weight gain, and upper and lower respiratory tract irritation. The responses were generally observed following exposure to the highest concentration, and not in the two lower concentrations. Considering the consistent observation of neurotoxic effects across experiments in rats and dogs, these effects following inhalation exposure to hexachloroethane were selected as the critical effect.

Methods of Analysis. Neurological effects were observed in male and non-pregnant female Sprague-Dawley rats, male Beagle dogs, and pregnant Sprague-Dawley rats only at the highest dose tested. Incidence data were not reported, which precluded application of BMD modeling. Therefore, the NOAEL of 465 mg/m^3 identified in Weeks et al. (1979) was selected as the POD for the derivation of the RfC based on effects in male and non-pregnant female rats and male dogs exposed to hexachloroethane for 6 weeks and pregnant rats exposed for 11 days, on GD 6–GD16.

I.B.3. UNCERTAINTY FACTORS

UF = 3000

An interspecies uncertainty factor, UF_A, of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans in the absence of information to characterize the toxicodynamic differences between rats and humans after oral HCE exposure. This value is adopted by convention, where an adjustment from an animal-specific POD_{ADJ} to a POD_{HEC} has been incorporated as described in the RfC methodology (U.S. EPA, 1994a).

An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to oral HCE exposure in the human population.

A subchronic-to-chronic, UF_S, of 10 was applied to account for extrapolation from a subchronic exposure duration study to a chronic RfD. The study selected as the principal study was a 6 week study by Weeks et al. (1979). No chronic inhalation studies were identified for HCE; therefore, there were no data to inform the effects that might be observed with increased exposure duration.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because this assessment utilized a NOAEL as the POD.

A database uncertainty factor, UF_D, of 10 was applied to account for deficiencies in the toxicity database for inhalation exposure to HCE. The toxicity data for inhalation exposure to HCE is limited and largely restricted to one subchronic (6-week) inhalation study (Weeks et al., 1979) in rats, male dogs, male guinea pigs, and quail. The same investigators performed a developmental/teratogenic study and an acute study (single 6 or 8 hour inhalation exposures) in rats. Although maternal toxicity was reported in the developmental/teratogenic study, fetuses of HCE-exposed dams did not exhibit any significant skeletal or soft tissue anomalies. The toxic effects observed in the dams in the developmental/teratogenic study (11-day exposure) were similar to those observed in the rats exposed for 6 weeks, although additional effects were observed in the rats exposed for the longer duration. The database lacks a long-term study and a multigeneration reproductive toxicity study. In addition, the database lacks studies of neurotoxicity and developmental neurotoxicity, endpoints of concern based on the available inhalation data demonstrating neurotoxicity in rats and dogs.

I.B.4. ADDITIONAL STUDIES/COMMENTS

The database of inhalation toxicity studies on hexachloroethane is limited. Human studies demonstrated hexachloroethane exposure in smoke bomb production workers, but the sample sizes were too small to reach definitive conclusions regarding health effects, and the exposure was likely a mixture of hexachloroethane and zinc oxide. There were no chronic inhalation studies available. The inhalation exposure database for hexachloroethane consisted of an acute study in rats (Weeks and Thomasino, 1978) and a subchronic inhalation study in four species that included a developmental/teratogenic toxicity experiment (Weeks et al., 1979). The database of inhalation toxicity studies on hexachloroethane is limited to a subchronic inhalation study and an acute exposure study. The subchronic inhalation study by Weeks et al. (1979), as the only repeated exposure study available, was selected as the principal study for the derivation of the RfC. An acute study of inhalation exposure in rats (Weeks and Thomasino, 1978) provided support for effects observed in the Weeks et al. (1979) subchronic studies.

Weeks and Thomasino (1978) exposed six male rats/concentration (strain not specified, although one table in the report indicated strain as Sprague-Dawley) to 2,500 or 57,000 mg/m^3 hexachloroethane for 8 hours and to 17,000 mg/m^3 hexachloroethane for 6 hours. Postexposure observations were carried out for 14 days. Male rats exposed for 8 hours to 2,500 mg/m^3 hexachloroethane displayed no toxic signs during exposure or for 14 days thereafter. Body weight gain was slightly, but not significantly reduced over the 14-day exposure period. Male rats exposed for 8 hours to 57,000 mg/m^3 hexachloroethane displayed

severe toxic signs including death. At 6 hours, one rat had a staggered gait. At 8 hours, 2/6 rats were dead. The surviving rats showed statistically significant reductions in mean body weight on exposure days 0 (7%), 1 (21%), 3 (19%), 7 (15%), and 14 (15%), compared with controls. Necropsy did not reveal any gross exposure-related lesions. Microscopy revealed that two of the four surviving rats had minimally to moderately severe subacute diffuse interstitial pneumonitis and vascular congestion. Additionally, a purulent exudate of the nasal turbinates was observed in one control and one treated rat. The authors concluded that this effect was not exposure-related, but rather was indicative of a low-grade endemic upper respiratory disease. The male rats exposed for 6 hours to 17,000 mg/m^3 showed slight reductions in body weight gain on postexposure days 1 (5%) and 3 (4%) and body weights similar to controls for the remaining 11 days of the postexposure period. Two of the six rats demonstrated a staggered gait. No exposure-related gross or histopathological changes were observed in tissues and organs.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF).

I.B.5. CONFIDENCE IN THE CHRONIC INHALATION RfC

Study — Low
Database — Low
RfC — Low

Overall confidence in the RfC is low. Confidence in the principal study, Weeks et al. (1979), is low. The 6-week study was conducted in several species (including male dogs, male and female rats, male guinea pigs, and quail). The study used three exposure groups (145, 465, and 2,517 mg/m^3) plus a control. The study was limited by the relatively short exposure duration (6 weeks) and minimal reporting of effects, especially quantitative changes. Confidence in the database is low because the database included one acute and one subchronic toxicity study in multiple species and one developmental toxicity study in rats. The database lacks studies by another laboratory and a multigenerational reproductive study.

For more detail on Dose-Response Assessments, exit to the toxicological review, Section 5 (PDF).

I.B.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC INHALATION RfC

Source Document – – *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name — Hexachloroethane
CASRN — 67-72-1
Section II. Last Revised — 09/23/2011

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated

concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

A previous cancer assessment for hexachloroethane was posted on the IRIS database in 1987. At that time, hexachloroethane was classified as a C carcinogen (possible human carcinogen), based on the observation of carcinomas in one mouse strain following oral exposure to hexachloroethane. An oral cancer slope factor (CSF) of 1.4×10^{-2} mg/kg-day was derived from the tumor incidence data for hepatocellular carcinoma in male and female $B6C3F_1$ mice exposed to hexachloroethane by gavage for 78 weeks, followed by an observation period of 12-13 weeks after cessation of exposure (NCI, 1978). The linearized multistage extra risk procedure was used for extrapolation. A drinking water unit risk of 4×10^{-7} per (µg/L) was derived. An inhalation unit risk (IUR) was not previously derived.

II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b), HCE is "likely to be carcinogenic to humans" based on evidence of statistically significant increased incidences of multiple tumor types in male rats and both sexes of mice (NTP, 1989; NCI, 1978). Specifically, NTP (1989) reported dose-dependent increases in the combined incidence of renal adenomas or carcinomas in male F344/N rats (see Table 4-6). NTP (1989) also reported increases in the incidence of pheochromocytomas in male F344/N rats, although the increase was not dose-related (see Table 4-7). NCI (1978) observed statistically significant increases in the incidence of hepatocellular carcinomas in male and female $B6C3F_1$ mice (see Table 4-10). The male mice demonstrated a dose-related increase in hepatocellular carcinomas, although increases in hepatocellular carcinomas in female mice were not dose-related.

Some data suggest that HCE-induced kidney tumors in male rats may involve a male rat-specific α_{2u}-globulin-mediated mode of action. As this mode of action is unique to the male rats, there is some uncertainty regarding the human relevance of these tumors for human health assessment. The available data on the role of α_{2u}-globulin-mediated mode of action in the carcinogenic effects of HCE were considered (see Section 4.7.3.1). EPA concluded that there is insufficient evidence to attribute HCE-induced kidney tumors in male rats to an α2u-globulin mode of action and that the mode of action for renal tumors is unknown.

The available data are considered insufficient to describe the mode of action for the carcinogenic effects of HCE in the liver (see Section 4.7.3.2). It is possible that the HCE-induced hepatocellular carcinomas in mice occur as a result of the binding of HCE metabolites

to liver macromolecules and the generation of free radicals during HCE metabolism. These processes could potentially lead to cytotoxicity, inflammation, and regenerative cell proliferation. However, these potential key events have not been evaluated for HCE.

The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (Greim et al., 2009; Powers et al., 2008) . Although more common in laboratory rats, evidence suggests that rat pheochromocytomas may have similarity to human pheochromocytomas and that they may be produced by the same mechanism of action (Greim et al., 2009; Eisenhofer et al., 2004; Lehnert et al., 2004; Elder et al., 2003; Goldstein et al., 1999) . Data are lacking to describe the mode of action for pheochromocytomas following HCE exposure (see Section 4.7.3.3).

The descriptor "likely to be carcinogenic to humans" is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "carcinogenic to humans." An example provided in the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) is "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." As is discussed in Section 4.2.1.2 of this assessment the results from several rodent bioassays indicate that HCE exposure can cause tumors in two species, both sexes of animals, and multiple sites. On this basis, these data support the cancer descriptor "likely to be carcinogenic to humans." However, there are uncertainties associated with relating the observed tumors in animals following exposure to HCE to human carcinogenicity. Additional mechanistic data, particularly related to the formation of the renal tumors in male rats, would inform the uncertainty associated with the assumption that these tumors are relevant to humans. If these tumors were determined to not be relevant to humans, then the weight of evidence regarding human carcinogenic potential would be reduced.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005b) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of HCE via the oral route demonstrated that tumors occurred in tissues remote from the site of absorption. Information on the carcinogenic effects of HCE via the inhalation and dermal routes in humans or animals was absent. Based on the observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it was assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, the data are sufficient to conclude that HCE is "likely to be carcinogenic to humans" by all routes of exposure.

For more detail on Dose-Response Assessments, exit to the toxicological review, Section 5 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF).

II.A.2. HUMAN CARCINOGENICITY DATA

There are no available studies on cancer in humans associated with exposure to hexachloroethane.

II.A.3. ANIMAL CARCINOGENICITY DATA

Two chronic oral exposure bioassays provided evidence of carcinogenic effects following hexachloroethane exposure in rats and mice. NTP (1989) provided evidence of renal adenomas and carcinomas, and pheochromocytomas and malignant pheochromocytomas, in male F344/N rats in a 2-year (103 weeks) cancer bioassay. NCI (1978) provided evidence of hepatocellular carcinomas in male and female $B6C3F_1$ mice in a 78-week cancer bioassay. Both NTP (1989) and NCI (1978) were well-designed studies, conducted in both sexes of two species with 50 animals/sex/dose. Each study utilized two dose groups of hexachloroethane and an untreated control group, with examination of a wide range of toxicological endpoints in both sexes of the rodents. Some limitations associated with the NCI (1978) study in mice included changes to the dosing regimen 9 weeks into the study, cyclical dosing periods, and decreased survival in all study groups for the male mice. Individual animal data were unavailable to perform time-to-tumor modeling or adjust the tumor incidences for survival before BMD modeling.

NTP (1989) conducted a chronic toxicity/carcinogenicity bioassay in F344/N rats. Groups of 50 male rats/dose were administered TWA doses of 7 and 14 mg/kg-day of hexachloroethane (purity >99%) by corn oil gavage, 5 days/week for 103 weeks. Groups of 50 female rats/dose were administered TWA doses of 57 and 114 mg/kg-day, by corn oil gavage, 5 days/week for 103 weeks. Male rats exhibited a dose-related, statistically significant increase in the incidence of combined renal adenomas or carcinomas at the highest dose. Combined renal adenomas or carcinomas were observed in 2, 4, and 14%, in controls (0), 7, and 14 mg/kg-day males, respectively. No hexachloroethane-related renal tumors were observed in female rats. The combined incidence of all three types of pheochromocytomas (benign, malignant, and complex pheochromocytomas) was significantly increased in males treated with 7 mg/kg-day hexachloroethane (62%) and increased in males treated with 14 mg/kg-day (43%) when compared with vehicle controls (30%) and historical controls in the study laboratory (75/300;

25 ± 7%) and in NTP studies (543/1,937; 28 ± 11%). No hexachloroethane-related adrenal gland tumors were observed in female rats.

NCI (1978; Weisburger, 1977) conducted a chronic toxicity/carcinogenicity bioassay in Osborne-Mendel rats. Hexachloroethane (purity >98%) at doses of 0, 250, or 500 mg/kg-day was administered by corn oil gavage to 50 rats/sex/dose for 5 days/week for 78 weeks. Following termination of exposure, rats were observed for 33–34 weeks for a total duration of 111–112 weeks. Twenty rats/sex were used for the untreated and vehicle controls. Starting in week 23, rats in the exposure groups began a 5-week cyclic rotation that involved 1 week without exposure followed by dosing for 4 weeks. After adjustment from 5 days/week for 78 weeks, with the 5-week cyclic rotation for part of the time, to continuous exposure over the standard 2 years for a chronic bioassay, the TWA doses were 113 and 227 mg/kg-day. Mortality was increased in the 113 and 227 mg/kg-day males with survival rates of 24/50 (48%) and 19/50 (38%), respectively, compared with 14/20 (70%) in the untreated controls. Survival rates for the female rats were 14/20 (70%) for both the untreated and vehicle controls, and 27/50 (54%) and 24/50 (48%) for the 113 and 227 mg/kg-day dose groups, respectively. All of the tumor types observed had been encountered previously as spontaneous lesions in the Osborne-Mendel rat strain, and no statistical differences in frequencies were observed between treated and control rats. NCI concluded that there was no evidence of carcinogenicity in this rat study. Notably, the doses used in the Osborne-Mendel rats of the NCI (1978) study were approximately 16 times greater than those doses administered to F344 male rats by NTP (1989).

NCI (1978; Weisburger, 1977) also conducted a chronic toxicity/carcinogenicity bioassay in $B6C3F_1$ mice. Hexachloroethane (purity >98%) was administered by corn oil gavage at TWA doses of 360 and 722 mg/kg-day for 5 days/week for 78 weeks, followed by 12–13 weeks of an observation period (total 91 weeks). Survival rates in males were 5/20 (25%), 1/20 (5%), 7/50 (14%), and 29/50 (58%) in the vehicle control, untreated control, and 360 and 722 mg/kg-day dose groups, respectively. Survival rates in females were 80, 85, 80, and 68% in vehicle control, untreated control, 360 and 722 mg/kg-day groups, respectively. Both male and female mice exhibited significantly increased incidences of hepatocellular carcinomas. The treated males demonstrated an increased tumor response for hepatocellular carcinomas that was dose-related: 30 and 63% in the 360 and 722 mg/kg-day dose groups, respectively, compared with 10% in pooled vehicle controls and 15% in matched vehicle controls. Females demonstrated an increased tumor response that was not dose related in that a higher incidence of hepatocellular carcinomas occurred at the low dose (40%) compared with the high dose (31%); pooled vehicle and matched vehicle controls had incidences of 3 and 10%, respectively. NCI concluded that hexachloroethane was carcinogenic in both sexes of $B6C3F_1$ mice.

II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

In addition to the two chronic bioassays in rodents, evidence of hexachloroethane–induced promotion potential (following treatment with DEN [N-diethylnitrosamine]), but not initiation potential, was observed in the liver of male Osborne-Mendel rats administered a single gavage dose of 497 mg/kg hexachloroethane (Milman et al., 1988; Story et al., 1986).

Lattanzi et al. (1988) reported in vivo and in vitro binding of hexachloroethane to DNA, RNA, and protein in mice and rats. In both rats and mice administered single i.p. injections of 127 µCi/kg [^{14}C]-hexachloroethane, in vivo covalent binding of hexachloroethane for RNA was consistently much greater than that for DNA or protein. DNA exhibited the lowest amount of hexachloroethane binding. Species differences were evident for all three macromolecule types (DNA, RNA, and protein), with the mouse exhibiting much higher levels (9 times greater) of covalent binding for DNA in the liver than the rat. The binding was 2 and 3 times greater for mice than rats with RNA and protein, respectively, from the liver. The binding was similar between species, but slightly greater in mice, for the kidney, lung, and stomach analyses. In vitro covalent binding to DNA was observed at comparable levels in liver microsomes from both rats and mice following exposure to hexachloroethane. Kidney microsomes from rats and mice produced significantly greater amounts of DNA binding compared with controls, with greater amounts of DNA binding from mice (threefold increase) compared with rats (twofold increase). Microsomes from the lungs and stomachs in both species did not display increased DNA binding activity over corresponding controls.

In vivo genotoxicity studies have not been performed in humans exposed to hexachloroethane. In vivo exposure to animals resulted in predominantly negative results. Similarly, in vitro genotoxicity studies conducted in microorganisms, cultured mammalian cells, and insects were largely negative both in the presence and absence of exogenous metabolic activation.

II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

II.B.1. SUMMARY OF RISK ESTIMATES

II.B.1.1. Oral Slope Factor: 4×10^{-2} per mg/kg-day

LED_{10}, lower 95% bound on exposure at 10% extra risk – 2.45 mg/kg-day
ED_{10}, central estimate of exposure at 10% extra risk – 3.74 mg/kg-day

The slope of the linear extrapolation from the central estimate ED_{10} is 0.1/(3.74 mg/kg-day) = 3×10^{-2} per mg/kg-day.

The slope factor for hexachloroethane should not be used with exposures exceeding the point of departure (POD) (2.45 mg/kg-day), because above this level, the fitted dose-response model better characterizes what is known about the carcinogenicity of hexachloroethane.

II.B.1.2. Drinking Water Unit Risk*: 1×10^{-6} per µg/L

Drinking Water Concentrations at Specified Risk Levels

Risk Level	Lower Bound on Concentration Estimate*
E-4 (1 in 10,000)	90 µg/L
E-5 (1 in 100,000)	9 µg/L
E-6 (1 in 1,000,000)	0.9 µg/L

*The unit risk and concentration estimates assume water consumption of 2 L/day by a 70 kg human.

II.B.1.3. Extrapolation Method

The multistage model with linear extrapolation from the POD (LED_{10})

II.B.2. DOSE-RESPONSE DATA

Tumor Type — Renal adenomas and carcinomas (combined)
Test animals — Male F344 rats
Route — Oral
Reference — NTP (1989)

Summary of incidence data in rodents orally exposed to hexachloroethane for use in cancer dose-response assessment

Study	Sex/strain/species	Endpoint	Hexachloroethane dose (mg/kg-day)	Incidence
NTP (1989)	Male F344 rats	Renal adenoma or carcinoma (combined)	0	1/50 (2%)
			7.1	2/50 (4%)
			14.3	7/50 (14%)[a]
NCI (1978)	Male B6C3F$_1$ mice	Hepatocellular carcinoma	0	3/20 (15%)[b]
			360	15/50 (30%)[a]
			722	31/49 (63%)[a]

[a]Denotes statistical significance.

[b]Incidence data are for the matched vehicle controls rather than the pooled controls from NCI (1978)

Summary of BMD modeling results for oral cancer assessments of hexachloroethane

Study	Sex/strain/species	Endpoint	"Best-fit" model	BMR	BMD_{10}	$BMDL_{10}$ or POD	Oral slope factor $(mg/kg\text{-}day)^{-1}$
NTP (1989)	Male F344 rats	Renal adenomas or carcinomas (combined)	2° Multistage	0.1	3.74	2.45	0.04
NCI (1978)	Male $B6C3F_1$ mice	Hepatocellular carcinomas	2° Multistage	0.1	38.09	13.80	0.007

II.B.3. ADDITIONAL COMMENTS

The multistage model was also fit to the incidences of pheochromocytomas or malignant pheochromocytomas in male rats and the incidences of hepatocellular carcinomas in male and female mice. The model exhibited a significant lack of fit for the pheochromocytomas and hepatocellular carcinomas in female mice (according to the X^2 statistic with $p < 0.1$). Thus, these datasets were not useful for dose response assessment because the tumor incidences are not a monotonic increasing function of dose, as demonstrated by the Cochran-Armitage Trend Test. Therefore, the renal adenomas/carcinomas (combined) in male rats (NTP, 1989) and the hepatocellular carcinomas in male mice (NCI, 1978) were used to derive candidate oral slope factors (see Table 5-6 of the Toxicological Review of Hexachloroethane).

The candidate oral slope factors were derived by linear extrapolation to the origin from the POD by dividing the BMR by the $BMDL_{10}$ (the lower bound on the exposure associated with a 10% extra cancer risk). The oral slope factor represents an upper bound estimate on cancer risk associated with a continuous lifetime exposure to hexachloroethane. In accordance with the U.S. EPA guidelines (2005a), an oral slope factor for renal tumors in male rats of 0.04/(mg/kg day) was calculated by dividing the BMR of 0.1 by the human equivalent $BMDL_{10}$ of 2.45 mg/kg day (Appendix B of the Toxicological Review of Hexachloroethane). An oral slope factor for hepatocellular tumors in male mice of 0.007per mg/kg day was

calculated by dividing the BMR of 0.1 by the human equivalent $BMDL_{10}$ of 13.80 mg/kg day (Appendix B of the Toxicological Review of Hexachloroethane). The rats exhibited greater sensitivity to hexachloroethane induced carcinogenicity than the mice. Thus, the risk estimate associated with the male rats that developed renal adenomas or carcinomas was selected as the oral slope factor of 0.04 per mg/kg day for hexachloroethane.

II.B.4. DISCUSSION OF CONFIDENCE

Relevance to humans. As described in Section 4.7.3 of the Toxicological Review, the modes of action for the kidney (adenomas/carcinomas) and adrenal gland tumors (pheochromocytomas) in male rats and liver tumors (hepatocellular carcinomas) in male and female mice are unknown. The human relevance of the renal tumor mode of action was considered in Section 4.7.3.1. An evaluation of the available data concluded that there were insufficient data to support an α_{2u}-globulin mode of action for the development of renal tumors. Additional information on key data gaps (e.g., immunohistochemical data identifying α_{2u}-globulin in the hyaline droplets, data on the incidence of end stage renal failure or high severe nephropathy for controls and hexachloroethane-exposed animals, presence of foci of atypical hyperplasia, and if the location of renal adenomas were within the areas of chronic progressive nephropathy) would inform the human relevance of the observed kidney tumors.

The human relevance of the liver tumor mode of action was considered in Section 4.7.3.2 of the Toxicological Review. Experimental animal literature demonstrated that oral exposure to hexachloroethane induces liver tumors in male and female mice. A potential mode of action for hexachloroethane-induced hepatocellular carcinomas in mice was the binding of hexachloroethane metabolites to liver macromolecules and the generation of free radicals during hexachloroethane metabolism, causing key events in the carcinogenic process such as cytotoxicity, inflammation, and regenerative cell proliferation. However, these potential key events have not been evaluated for hexachloroethane. Additional data distinguishing the similarities and differences between experimental animals and humans in terms of hexachloroethane metabolism or toxicity would inform the human relevance of the reported liver tumors.

The human relevance of the adrenal gland tumor mode of action was considered in Section 4.7.3.3 of the Toxicological Review. Pheochromocytomas occur in both humans and rats, although they are more common in laboratory rats. Evidence suggests that certain rat pheochromocytomas may have similarity to human pheochromocytomas (Powers et al., 2008). Furthermore, mechanisms of action inducing pheochromocytomas in rats are expected to occur in humans as well (Greim et al., 2009). The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (Greim et al., 2009; Powers et al., 2008). Additional data distinguishing the similarities and

differences between pheochromocytoma induction in animals and humans would inform the human relevance of the reported adrenal gland tumors.

In the absence of information indicating otherwise, the kidney and adrenal gland tumors in male rats and liver tumors in male and female mice are considered relevant to humans.

Choice of low-dose extrapolation approach. The mode of action is a key consideration in clarifying how risks should be estimated for low-dose exposure. In the absence of mode of action information to inform the dose-response at low doses, a linear-low-dose extrapolation approach was used to estimate human carcinogenic risk associated with hexachloroethane exposure. The extent to which overall uncertainty in low-dose risk estimation would be reduced if the mode of action for hexachloroethane was characterized is not known.

Etiologically different tumor types were not combined across organ sites prior to modeling, to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors. The human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.007 to 0.04 per mg/kg-day, a range less than one order of magnitude, with greater risk coming from the male rat kidney data.

Interspecies extrapolation. An adjustment for cross-species scaling ($BW^{0.75}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the U.S. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Choice of model. There are no human data from which to estimate human cancer risk; therefore, the risk estimate must rely on data from studies of rodents exposed to levels greater than would occur from environmental exposures. Without human cancer data or additional mechanistic data, the human relevance of the rodent cancer results is uncertain. The occurrence of increased incidences of kidney and adrenal gland tumors in male rats, and liver tumors in male and female mice exposed to hexachloroethane from the oral route of exposure suggested that hexachloroethane is potentially carcinogenic to humans. However, the lack of concordance in tumor sites between the two rodent species makes it more difficult to quantitatively estimate human cancer risk.

Regarding low-dose extrapolation, in the absence of mechanistic data for biologically based low-dose modeling or mechanistic evidence supporting a nonlinear approach, a linear low-dose extrapolation was carried out from the $BMDL_{10}$. It is expected that this approach provides an upper bound on low-dose cancer risk for humans. The true low-dose risks cannot be known without additional data.

With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range (U.S. EPA, 2005) and extrapolating from a POD in the observable range is designed in part to minimize model dependence. Measures of statistical uncertainty require assuming that the underlying model and associated assumptions are valid for the data under consideration. The multistage model used provided an adequate fit to all the datasets for kidney and liver tumors. For the multistage model applied to the incidence of tumors, the BMDL values should generally be within a factor of 3 of the BMDs. This indicates that there is a reasonably typical degree of uncertainty at the 10% extra risk level. A large difference between the BMD and BMDL raises concern that the algorithm for the calculation of the BMDL is not accurate (U.S. EPA, 2005). The ratios of the BMD_{10} values to the $BMDL_{10}$ values did not exceed a value of 2.6, indicating that the estimated risk was not influenced by any unusual variability in the model and associated assumptions.

Dose metric. Hexachloroethane is potentially metabolized to PERC and pentachloroethane; however, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity and carcinogenicity of hexachloroethane. If the actual carcinogenic moiety(ies) is(are) proportional to administered exposure, then use of administered exposure as the dose metric provides an unbiased estimate of carcinogenicity. On the other hand, if administered exposure is not the most relevant dose metric, then the impact on the human equivalent slope factor is unknown. Consequently; the low-dose cancer risk value may be higher or lower than that estimated, by an unknown amount. In the absence of data identifying the carcinogenic moiety for hexachloroethane, the administered exposure was selected as the dose metric.

Bioassay selection. Of the two chronic animal bioassays selected for BMD analysis and subsequent quantitative cancer assessment, the NTP (1989) study was used for the development of an oral slope factor because male rats exhibited greater sensitivity to hexachloroethane-induced carcinogenicity than mice.

Choice of species/gender. The oral slope factor for hexachloroethane was quantified using the tumor incidence data for male rats, which were found to be more sensitive than male or female mice to the carcinogenicity of hexachloroethane. The oral slope factor calculated from male rats was higher than the slope factors calculated from male and female mice. As there is no information to inform which species or gender of animals would be most applicable to humans, the most sensitive group was selected for the basis of the oral slope factor. Evidence suggesting the kidney is a target organ of hexachloroethane toxicity in both species lends strength to the concern for human carcinogenic potential.

Human population variability. The extent of interindividual variability or sensitivity to the potential carcinogenicity of hexachloroethane is unknown. There are no data exploring whether there is differential sensitivity to hexachloroethane carcinogenicity across life stages. In addition, neither the extent of interindividual variability in hexachloroethane metabolism nor human variability in response to hexachloroethane has been characterized. Factors that could contribute to a range of human responses to hexachloroethane include variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of hexachloroethane or antioxidant protection systems. This lack of understanding about potential susceptibility differences across exposed human populations thus represents a source of uncertainty. Humans are expected to be more genetically heterogeneous than inbred strains of laboratory animals (Calderon, 2000), and this variability is likely to be influenced by ongoing or background exposures, diseases, and biological processes.

II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

In the absence of data on the carcinogenicity of hexachloroethane via the inhalation route, an inhalation unit risk has not been derived.

II.C.1. SUMMARY OF RISK ESTIMATES

Not applicable.

II.C.2. DOSE-RESPONSE DATA

Not applicable.

II.C.3. ADDITIONAL COMMENTS

Not applicable.

II.C.4. DISCUSSION OF CONFIDENCE

Not applicable.

II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

II.D.1. EPA DOCUMENTATION

Source Document — *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Hexachloroethane* (U.S. EPA, 2011). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Completion Date — 09/23/2011

II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

III. [reserved]
IV. [reserved]
V. [reserved]

VI. BIBLIOGRAPHY

Substance Name — Hexachloroethane (HCE)
CASRN — 67-72-1

VI.A. ORAL RFD REFERENCES

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VI.C. CARCINOGENICITY ASSESSMENT REFERENCES

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VII. Revision History

Substance Name — Hexachloroethane (HCE)
CASRN — 67-72-1
File First On-Line 03/31/1987

Date	Section	Description
09/23/2011	I, II, VI, VIII	RfD and cancer assessments updated. RfC added.

VIII. SYNONYMS

Substance Name — Hexachloroethane (HCE)
CASRN — 67-72-1
Section VIII. Last Revised — 09/23/2011

- 67-72-1
- AVLOTHANE
- CARBON HEXACHLORIDE
- CARBON TRICHLORIDE
- DISTOKAL
- DISTOPAN
- DISTOPIN
- EGITOL
- ETHANE HEXACHLORIDE
- ETHYLENE HEXACHLORIDE
- FALKITOL
- FASCIOLIN
- HEXACHLOR-AETHAN
- HEXACHLOROETHANE
- 1,1,1,2,2,2-HEXACHLOROETHANE
- HEXACHLOROETHYLENE
- MOTTENHEXE
- NA 9037
- NCI-C04604
- PERCHLOROETHANE
- PHENOHEP

- RCRA WASTE NUMBER U131