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Provisional Peer Reviewed Toxicity Values for

Hexachlorobutadiene
(CASRN 87-68-3)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

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

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

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR HEXACHLOROBUTADIENE (CASRN 87-68-3)

Background

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

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

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

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

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

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

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

Disclaimers

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

circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

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

Questions Regarding PPRTVs

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

INTRODUCTION

No reference dose (RfD) or reference concentration (RfC) values are available for hexachlorobutadiene (HCBD) in the Integrated Risk Information System (IRIS) database (U.S. EPA, 2007). The Health Effects Assessment Summary Table (HEAST) lists a chronic oral RfD of 2E-4 mg/kg-day and no subchronic RfD (U.S. EPA, 1997). The source documents referenced for the RfD value in HEAST included a 2-year dietary study in rats (Kociba et al., 1977) and a 13-week dietary study in mice (NTP, 1991; Yang et al., 1989). The chronic oral RfD value cited in HEAST was derived from a LOAEL of 0.5 mg/kg-day, based on renal tubule regeneration observed in a 13-week dietary study in mice (NTP, 1991; Yang et al., 1989). The Drinking Water Standards and Health Advisories also includes an RfD of 2E-4 mg/kg-day for HCBD (U.S. EPA, 2004). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994) identifies a Health Effects Assessment (HEA) (U.S. EPA, 1984) and a Drinking Water Health Advisory report (U.S. EPA, 1987). No oral or inhalation RfD values were provided in the HEA (U.S. EPA, 1984). An RfD value of 0.002 mg/kg-day was calculated for use in the derivation of the drinking water equivalent level (DWEL) (U.S. EPA, 1987), based on kidney effects observed in the 2-year dietary study in rats (Kociba et al., 1977). A no-observed-adverse-effect level (NOAEL) of 0.2 mg/kg-day was identified from this study, based on functional and histopathological changes in the kidney, and a composite uncertainty factor (UF) of 100 was applied to account for interspecies and interindividual differences.

An Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for HCBD (ATSDR, 1994) derived an intermediate-duration oral Minimal Risk Level (MRL) of 0.0002 mg/kg-day, based on the presence of kidney damage in female mice from a 13-week

dietary study (NTP, 1991). A lowest-observed-adverse-effect level (LOAEL) value of 0.2 mg/kg-day was identified, based on tubular cell degeneration and regeneration in the renal cortex, and a composite uncertainty factor (UF) of 1000 was applied to derive the intermediate-duration oral MRL (factors of 10 each to account for the interindividual variation in the human population, the uncertainty in extrapolating animal data to the case of human and uncertainty in using LOAEL data rather than NOAEL data). Because renal tubular hyperplasia was observed at 2 mg/kg-day in a chronic dietary study in rats (Kociba et al., 1977) and no effect was seen at 0.2 mg/kg-day in this study (the LOAEL for kidney effects in the 13-week mouse study), the intermediate-duration MRL was considered protective for chronic exposures and a chronic MRL was not proposed. Inhalation MRL values were not derived by ATSDR for HCBD due to the lack of sufficient data to identify a target organ or reliable NOAEL values (ATSDR, 1994). Occupational exposure standards and guidelines for HCBD, based on skin irritation and kidney effects, include American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value–time-weighted average (TLV-TWA) and National Institute for Occupational Safety and Health (NIOSH) TWA values of 0.02 ppm (0.24 mg/m^3) (ACGIH, 2005; NIOSH, 2005). An Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) value is not available for HCBD (OSHA, 2006).

A cancer assessment for HCBD is available on IRIS (U.S. EPA, 2007), in the HEAST (U.S. EPA, 1997), and on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). HCBD is considered to be a possible human carcinogen (Group C) based on kidney tumors observed in male and female rats from one study. An oral slope factor of 0.078 $(mg/kg-day)^{-1}$ was derived, based on renal tubular adenomas and adenocarcinomas observed in rats given HCBD in the diet (Kociba et al., 1977). An inhalation unit risk value of $2.2x10^{-5}$ $(\mu g/m^3)^{-1}$ was calculated based on route extrapolation from the oral data (U.S. EPA, 2007). The International Agency for Research on Cancer (IARC) assigned HCBD to Group 3 (not classifiable as to its carcinogenicity to humans), based on limited evidence for the carcinogenicity of HCBD in animals and inadequate evidence in humans (IARC, 1999). The World Health Organization (WHO) Environmental Health Criteria document (WHO, 1994) also indicated that there was limited evidence for carcinogenicity of HCBD in animals and insufficient evidence in humans. HCBD was not included in the NTP (2005) 11^{th} Report on Carcinogens.

Literature searches were performed for the time period of 1965 to May, 2006 in TOXLINE, MEDLINE (plus PubMed cancer subset) and DART/ETICBACK. An update search of the TOXCENTER (BIOSIS) database was performed for the time period of 2000 to May, 2006. Databases searched without date limitations included TSCATS, RTECS, GENETOX, HSDB and CCRIS. Search of Current Contents encompassed November 2005 to May 2006.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure. No data were located regarding the oral toxicity or carcinogenicity of HCBD in humans.

Inhalation Exposure. Very little information pertaining to effects of inhalation of HCBD in humans is available. Howse et al. (2001) investigated biomarkers of early renal dysfunction in a cohort of subjects exposed to HCBD. This study was presented as an abstract only and few details were provided regarding the subject cohort or the nature of the exposure to HCBD. Urinary markers of renal disease were evaluated in 70 subjects known to be environmentally exposed to HCBD. Twenty-five subjects were eventually eliminated from consideration due to age, preexisting renal disease, medication use or exposure to other nephrotoxic compounds. The parameters investigated for the remaining 45 subjects included urinary albumin, total protein, γ-glutamyl transpeptidase (GGT), N-acetyl-β-glucosaminidase (NAG), leucine aminopeptidase (LAP), α- and π-glutathione transferases (GST) and retinol binding protein (RBP). Results were compared to the laboratory reference range for healthy workers. Urinary abnormalities occurred in 21 subjects, with 11 subjects exhibiting 2 or more abnormal tests. The most common effects were seen with the tubular markers LAP, GGT and α- and π-GST. No further information was provided.

Driscoll et al. (1992) carried out a study investigating liver dysfunction in workers exposed to a variety of chlorinated solvents (mainly carbon tetrachloride and perchlorethylene) and HCBD at a solvent production plant. The study included all 53 members of the workforce, but a number of individuals were excluded from the analysis because their blood samples were inadequate (6 individuals), they had not fasted before the blood samples were taken (11 individuals) or were taking antibiotics (1 individual). This left 35 subjects who were included in the analysis. Workers were categorized in relation to both HCBD exposure and overall solvent exposure at the plant. The results of repeated environmental monitoring in the plant were used to assign each worker to one of four classes of exposure to HCBD (0.0, 0.005, 0.01 or 0.02 ppm). Overall solvent exposure for all workers was low (less than 1 ppm), but varied with task; routine monitoring data from the plant records were used to assign workers to either a "lower" or "higher" solvent exposure category. Workers assigned to the various categories were similar in age and duration of employment.

Blood samples were collected from each worker after an overnight fast (Driscoll et al., 1992). Serum bile acids were assayed by high performance liquid chromatography and compared for each group. Standard tests for liver function [serum protein, albumin and bilirubin concentrations and alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GGT activities] were also carried out. Total bile acids were not significantly increased in relation to HCBD exposure, but a positive exposure-effect relationship with HCBD concentration was found for three individual bile acids (deoxycholic acid, glycine deoxycholic acid, taurine chenodeoxycholic acid) and for total deoxycholate (this includes deoxycholic acid and glycine deoxycholic acid). Using multiple linear regression and controlling for age and overall solvent exposure, these parameters had significant positive log-linear relations with exposure to HCBD. With respect to overall solvent exposure, there was no significant positive relationship for total bile acids or any individual bile acids (the researchers suggested that significant negative relationships with glycine deoxycholic acid, taurine cholate and total cholate may have resulted from misclassification of exposure for some workers).

Although liver function tests did not show any significant relationship with exposure to either HCBD or solvents overall in the Driscoll et al. (1992) study, serum bile acid

concentrations may be a more sensitive indicator of liver damage than standard tests of hepatic function. Franco et al. (1986) compared liver function in workers occupationally exposed to a mixture of organic solvents and an unexposed control group. The results from conventional tests of hepatic function were compared with those of the serum bile acid test, and the researchers concluded that the serum bile acid test had a higher sensitivity for the detection of liver dysfunction for the solvent mixture tested. The bile acid test results of Driscoll et al. (1992) suggest that exposure to HCBD may affect liver function. However, there was no supporting evidence for hepatotoxicity from standard liver function tests, workers were exposed to multiple solvents, the study did not include a control group of individuals unexposed to any solvents and the study did not assess possible confounders, such as previous hepatic disease and alcohol intake.

The only other available study of effects of HCBD in humans is a study carried out in Russia (Krasniuk et al., 1969). Krasniuk et al. (1969) recorded multiple toxic effects in vineyard workers seasonally exposed to HCBD (0.8-30 mg/m^3) and polychlorobutane-80 (0.12-6.7 mg/m^3) in the air over fumigated areas. A total of 205 workers were examined medically; 153 workers had 4 years of exposure to HCBD and polychlorobutane-80, while 52 workers had worked under the same conditions without exposure to the chemicals. The study reported multiple toxic effects in exposed workers, including the development of hypotension, cardiac disease, chronic hepatitis and disturbance of nervous function. The effects, however, are not well documented and cannot be attributed solely to HCBD.

Animal Studies

Oral Exposure.

Jonker et al., 1993 — A 4-week dietary study was conducted in Wistar rats (five/sex/group, 10 controls/sex) fed HCBD (98% pure) at concentrations of 0, 25, 100 or 400 ppm (Jonker et al., 1993). Using reference values for body weight and food consumption in Wistar rats from a subchronic study (male body weight 0.217 kg, female body weight 0.156 kg, male food consumption 0.02 kg/day, female food consumption 0.016 kg/day) (U.S. EPA, 1988), daily dose estimates were calculated to be 0, 2.3, 9.2 or 37 mg/kg-day for male rats and 0, 2.6, 10.2 or 41 mg/kg-day for female rats. During the 4^{th} week of the study, rats were deprived of water for 24 hours and food for 16 hours. Urine was collected during the last 16 hours of water deprivation and urine volume and density were measured. Urine samples were also visually inspected and analyzed for pH, protein, glucose, ketones, occult blood, urobilinogen and bilirubin. Urine samples were centrifuged and the sediment was examined microscopically. Hematology parameters, including hemoglobin, packed cell volume, red blood cells (RBCs) and total white blood cells (WBCs), were evaluated for tail vein blood samples that were obtained during the 4^{th} week of the study. Blood samples obtained at necropsy were analyzed for serum AP, AST and ALT activities, protein, albumin, bilirubin, urea, creatinine, inorganic phosphorous, calcium, sodium and potassium. The organ weights of the kidneys, adrenals and liver were recorded at necropsy and kidney tissue was prepared for histopathological evaluation.

Growth retardation and decreased food and water consumption were observed in male and female rats exposed to 100 or 400 ppm. Mean body weights measured on day 28 were

reduced by 34% in both male and female rats given 400 ppm HCBD in the diet. At 100 ppm, 28-day body weights were decreased by 10% in male rats and 15% in female rats. Increased volume and decreased density of the urine were observed in male rats receiving 100 ppm only. An increase in urinary epithelial cells was seen at 100 and 400 ppm HCBD in male and female rats and urinary ketones were increased at 400 ppm HCBD in both males and females. Clinical chemistry findings demonstrated increased AST activity (400 ppm males and females, 46% and 22% increased respectively), decreased total protein and albumin (400 ppm males only, 5% decrease), decreased urea (all female treatment groups, maximal decrease of 34%; 400 ppm males, 25% decrease), decreased creatinine (400 ppm females, 12% decrease), increased total bilirubin (6.7-fold and 2.4-fold increase in 400 ppm males and females, respectively) and decreased calcium (400 ppm males, 8% decrease). HCBD treatment resulted in a 13% decrease in absolute kidney weight in high-dose (400 ppm) male rats. Absolute kidney weights were similar to controls for all other treatment groups. An increase in relative kidney weight (organ:body weight ratio) was seen in male and female rats given 100 or 400 ppm HCBD (12 and 31% increase for male rats; 21 and 40% increase for female rats). The absolute organ weight of the adrenals was decreased in female rats given 100 or 400 ppm, while the relative adrenal weight was increased in high-dose male rats. Absolute liver weight was decreased in male rats at 400 ppm and in female rats at 100 and 400 ppm. Relative liver weight was increased in male rats given 100 ppm HCBD only. Relative organ weight increases (kidney, adrenals, and liver) are likely due to the observed decreases in body weight in male and females rats exposed to 100 ppm or 400 ppm HCBD. Kidney histopathology evaluation showed diffuse tubular cytomegaly (females at 100 ppm, males and females at 400 ppm) and focal nephrosis (males at 400 ppm). Incidence data for these lesions were not provided. Histopathological changes in the kidney were further described for a separate group of male and female rats given 100 ppm HCBD in the diet for 4 weeks. In female rats, necrosis, karyomegaly, hypercellularity and variable nuclear size were observed in inner cortex (incidence of 5/5 treated rats, 0/10 controls). NOAEL and LOAEL values of 25 and 100 ppm (2.6 and 10.2 mg/kg-day, respectively) were derived from this study, based on the kidney histopathology data in female Wistar rats.

NTP, 1991; Yang et al., 1989 — Dietary studies with HCBD were conducted in B6C3F_1 mice (NTP, 1991; Yang et al., 1989). In a 2-week study, mice (five/sex/group) received diets containing 0, 30, 100, 300, 1000 or 3000 ppm for 15 days. Animals were observed twice daily and were weighed initially and on days 7 and 15. Food consumption was measured on day 3 and every 2 days thereafter. Necropsy was performed on all animals and histopathology was evaluated in bone marrow, kidneys and liver for animals in the control, 300, 1000 and 3000 ppm groups. Organ weights were measured for the liver thymus kidneys, heart, brain, lung and testis.

All mice that were fed 1000 or 3000 ppm HCBD died before the end of the study. Growth retardation was observed in all HCBD treatment groups. Terminal body weights were 10%, 17% and 20% lower than controls for the 30, 100, and 300 ppm treatment groups, respectively. Control mice gained an average of 2.2 g over the course of the study, while mice given 30 ppm HCBD did not gain weight and mice given 100 ppm and 300 ppm experienced an average weight loss of 1.7 g and 2.1 g, respectively. The study authors indicated that it was unclear whether the observed growth retardation was treatment-related, due to the variability in the measured food consumption caused by scattering of feed by mice in the treatment groups. Daily dose estimates were calculated by the study authors based on feed consumption and body

weight measurements. Dietary concentrations of 0, 3, 30 and 300 ppm resulted in dose estimates by the study authors of 0, 3, 12 and 40 mg/kg-day in male mice and 0, 5, 16 and 49 mg/kg-day in female mice. Lower dietary intakes were reported for the 1000 and 3000 ppm dose groups (19 and 24 mg/kg-day in males; 30 and 36 mg/kg-day in females) due to the decreased food consumption occurring in these dose groups.

Clinical signs of toxicity were seen in mice given dietary concentrations ≥300 ppm. Lethargy, rough hair coat, hunched position and incoordination were observed. Decreased organ weights were seen in male and female mice from the 300 ppm dose group (28-49% decrease in thymus weight, 69-75% decrease in heart weight). Although the report does not indicate whether organ weight decreases were absolute or relative to bodyweight, the study authors suggested that the reduced organ weights were the result of stress and growth retardation and may be only secondarily related to HCDB treatment. Kidney lesions were observed in mice from each HCBD treatment group examined (300, 1000 and 3000 ppm). Severe necrosis of the cortex and outer medulla of the kidney was seen in mice from the 1000 and 3000 ppm dose groups that died prior to the end of the study. The necrosis was less severe at 300 ppm and regeneration was evident, especially in the pars recta (outer stripe of the outer medulla). Other lesions were seen in mice from the two highest dose groups, including lymphoid necrosis and atrophy in the spleen, thymus and lymph nodes, atrophy and necrosis of the red pulp of the spleen, testicular degeneration, and vacuolization and necrosis of hepatocytes. Minimal to mild depletion of the bone marrow (decrease in hematopoietic cells) was observed in mice treated with dietary concentrations of ≥300 ppm HCBD. NOAEL and LOAEL values were not identified from the 2-week study because histopathology evaluation was not performed for rats receiving 30 and 100 ppm HCBD.

In the 13-week dietary study, concentrations of 0, 1, 3, 10, 30 or 100 ppm HCBD (98% pure) were made available mixed in feed to 10 mice/sex. Body weights and food consumption rates were measured weekly. As reported by the authors, the average daily doses of HCBD were estimated to be 0, 0.1, 0.4, 1.5, 4.9 or 16.8 mg/kg-day in males and 0, 0.2, 0.5, 1.8, 4.5 or 19.2 mg/kg/day in females, when food consumption and body weight data were taken into account. Mice were observed twice daily and necropsy and histopathological evaluation of the kidneys was performed for all animals. Complete histopathology evaluation of the full range of organs and tissues was conducted for control and high-dose mice (100 ppm), and for those animals dying before the end of the study. Organ weights were measured at necropsy and samples were taken for a sperm count and motility evaluation, and for an analysis of vaginal morphology and cytology.

Although no clinical signs were evident in any of the animals in the study, one male mouse (1 ppm) died before the end of the study. HCBD treatment caused a decrease in the mean body weight in the two highest-dose groups of male mice and in the highest dose group in female mice throughout most of the study. Terminal mean body weights were 10 and 16% lower than controls for male mice in the 30 and 100 ppm dose groups, respectively, and 15% lower than controls for female mice exposed to 100 ppm HCBD. No major differences in food consumption were noted among treatment groups, suggesting that growth retardation is a toxic effect of HCBD. Absolute kidney weights were reduced (up to 24%) compared with controls in the three highest-dose male groups and the highest-dose female group (23%). Relative kidney weight was also decreased in these treatment groups (up to 19%) compared with controls. A 12% reduction

in absolute heart weight was also evident in the 100 ppm males (relative organ weight not reported). The principal histopathological finding was a compound-related increase in regeneration in the renal tubular epithelium that was most evident in the outer stripe of the outer medulla and extended into the medullary rays (pars recta) (see Table 1). Basophilic staining of the tubular cell cytoplasm and occasional mitoses were seen in regenerative cells. The necrosis that was evident at 300 ppm and above in the 2-week study was not seen after 13 weeks of exposure to 100 ppm or lower concentrations. Sperm motility was reduced in all dose groups, but the magnitude of this effect was not dose-related. No significant changes were seen in sperm count, incidence of abnormal sperm, estrous cyclicity or average length of the estrous cycle. NOAEL and LOAEL values of 0.2 and 0.5 mg/kg-day (1 and 3 ppm) were derived from this study, based on kidney lesions (renal tubule regeneration) in female rats exposed to HCBD in the diet for 13 weeks.

Table 1. The Incidence of Renal Tubule Regeneration in B6C3F$_1$ Mice Exposed to HCBD in the Diet for 13 Weeks (NTP, 1991; Yang et al., 1989)

	Incidence					
	0 ppm	**1 ppm**	**3 ppm**	**10 ppm**	**30 ppm**	**100 ppm**
Males	0/10	0/10	0/10	0/9	10/10[a]	10/10[a]
Females	0/10	1/10	9/10[a]	10/10[a]	10/10[a]	10/10[a]

[a]$p<0.05$, Fisher's Exact test performed for this analysis

Field et al. (1990) — Field et al. (1990) fed pregnant female CD rats (8-9/group) diets containing HCBD (98% purity) at concentrations of 0, 100, 200, 400, 750, 1100 or 1500 ppm on gestation day (GD) 17 through postnatal day (PND) 10. Animals were observed twice daily for clinical signs and were weighed on GD 0, 6, 11, 16 and 17 through PND 10. The reproductive and developmental parameters evaluated included litter size, sex ratio, pup body weights and percentage survival. On PND 4, litters were culled to 10 with an equivalent sex ratio, if possible. Pups were counted and weighed on PND 4, 7 and 10. On PND 10, one pup of either sex from each litter was randomly selected for urine and blood collection. Urine and blood samples were tested for glucose, urea, creatinine or total protein and osmolality was measured in the urine collected immediately following removal from the dam. One additional rat of each sex from each of the five litters was selected on PND 10 to undergo a "hydropenic test" as an indicator of renal competence. In this test, urine samples collected 4 and 6 hours following isolation from the dams were tested for osmolality. Pups were euthanized on PND 10 and liver and kidneys were weighed and prepared for histopathology. In the dams, samples of milk were collected from three dams/group, and liver and kidney tissues were weighed and processed for histopathological examination.

All dams receiving chow containing 1500 ppm HCBD became moribund and had to be terminated prematurely. Similarly, all animals (and their pups) exposed to 1100 ppm HCBD had to be terminated between PND 1 and PND 3. Clinical signs of toxicity in the two highest dose groups included excessive urination, alopecia, nasal discharge, redness of paws, tremors, piloerection, urogenital discharge, hindlimb weakness, lethargy and rough coat. Maternal body weight was decreased in all treatment groups above 100 ppm. Dams given a dietary concentration of 100 ppm HDBD had body weights similar to controls. On PND 10, maternal

body weights were decreased by 11, 21 and 31% in the 200, 400 and 750 ppm treatment groups, respectively. These reductions were accompanied by decreased food consumption during the gestational exposure period, compared with controls (43% decrease at 200 and 400 ppm and 73% decrease at 750 ppm). The study authors calculated an estimate of the daily dose using the food consumption rates for GD 20. Dose estimates of 0, 12, 22.5, 35.3 or 52.2 mg/kg-day were associated with dietary concentrations of 0, 100, 200, 400 or 750 ppm HCBD. The intake of HCBD throughout the study was considered to be variable, due to the fluctuation in food consumption. The HCBD content of maternal milk was shown to increase with increasing dietary concentration, when measured on PND 10. Relative kidney weight in dams was increased 25, 25, 44 and 78% above controls in rats from the 100, 200, 400 and 750 ppm HCBD treatment groups, respectively. Absolute kidney weights were not reported. Histopathological findings in dams demonstrated tubular regeneration of the pars recta of the proximal tubules in all treatment groups, with severity of the lesions being dose-related. At the higher dose levels, tubules were occasionally distended, appearing either empty or full of cell debris.

Three out of nine dams receiving 750 ppm HCBD delivered only dead pups, and, as a percentage, fewer pups from this group survived to PND 10 compared with controls (73% survival). In general, pups displayed dose-dependent reductions in body weight compared with controls, with those at the highest dose (750 ppm) displaying marked emaciation. Pup body weights on PND 10 were 94, 90, 59 and 51% of control pups for the 100, 200, 400 and 750 ppm HCBD treatment groups, respectively (statistical analysis not reported). Clinical chemistry results for the treated pups were similar to control. Following fluid deprivation, urine osmolality was increased in all HCBD-treated groups of dams and pups. Relative kidney weight in pups was increased by 8, 6, 12 and 21% above the control value for the 100, 200, 400 and 750 ppm HCBD treatment groups, respectively (statistical analysis was not reported). Absolute kidney weight was not reported. Histopathology examination showed kidney lesions in pups from the high-dose group only. The primary morphological changes were reduced kidney size and retention of the subscapular metanephric blastemal zone, which was considered by the study authors to reflect a delay in the postnatal development of the kidneys and apparent dehydration. The daily intake of HCBD in pups on PND 10 was calculated to range from 3 to 7% of the dose received by dams in the same dose group. The lowest dose tested (12 mg/kg-day, 100 ppm) is a LOAEL for maternal effects on the kidney (increased relative kidney weight, tubule regeneration). A NOAEL was not identified for maternal effects in this study. Effects in the offspring occurred at higher doses, with NOAEL and LOAEL values of 22.5 and 35.3 mg/kg-day (200 and 400 ppm), based on reduced pup body weight and increased relative kidney weight.

Stott et al., 1981 — Male Sprague-Dawley rats (4-6/group) were given 0, 0.2 or 20 mg/kg-day HCBD by oral gavage in corn oil for 21 consecutive days. An osmotic pump loaded with ^{3}H-thymidine was implanted 7 days prior to the end of the experiment and the rate of in vivo DNA synthesis was measured. Body weight gain was determined (frequency of measurement not indicated) and kidney weight was recorded at necropsy. Tissue samples were obtained from the central portion of the animal's left kidney and evaluated for histopathology. Rats were also given a single dose of ^{3}H-HCBD (20 mg/kg-day only) and were sacrificed 4 hours later for determination of in vivo renal DNA repair and DNA alkylation. In vitro studies conducted using HCBD included reverse mutation in *Salmonella typhimurium* and unscheduled DNA synthesis in primary rat hepatocytes.

In rats given 20 mg/kg-day for 3 weeks, body weight was decreased by 44%, kidney to body weight ratio was increased 1.3-fold, and a 1.8-fold increase was observed in the rate of renal DNA synthesis in vivo (not statistically significant due to high variability between animals). Histopathological lesions were also observed in rats from this group, occurring in the tubular epithelial cells of the inner and middle cortex. Lesions were characterized as degenerative and regenerative changes and included loss of cytoplasm, nuclear pyknosis, increased basophilia, mitotic activity and increased cellular debris located within the tubular lumen. No changes were observed in rats given 0.2 mg/kg-day (NOAEL value). The LOAEL for this study was 20 mg/kg-day.

Renal DNA repair was increased 1.27-fold and 1.54-fold (two trials) in rats given in a single oral dose of 20 mg/kg-day HCBD, as compared to controls. DNA alkylation was also observed in these rats. HCBD did not cause mutagenicity in Salmonella or unscheduled DNA synthesis in isolated rat hepatocytes.

Harleman and Seinen (1979) — Harleman and Seinen (1979) conducted a 2-week dietary study, a dietary reproduction study and a 13-week oral gavage study to evaluate the potential toxicity of HCBD in Wistar rats. In the 2-week dietary study, rats (24/sex/group) were exposed to 0, 50, 150 or 450 ppm HCBD in the diet for 14 days. Using reference values for body weight and food consumption in weanling Wistar rats (male body weight 0.053 kg, female body weight 0.052 kg, food consumption of 0.008 kg/day for both males and females) (U.S. EPA, 1988), daily dose estimates were calculated to be 0, 8, 23 or 68 mg/kg-day for male rats and 0, 8, 23 or 69 mg/kg-day for female rats. Body weights were measured at the beginning and end of the study. Liver and kidney weights were recorded at necropsy and histopathology of these organs was evaluated. Body weight was decreased in all HCBD treatment groups of female rats (10-33% decrease) and in the two highest dose groups of male rats (21 and 31% decrease at 150 or 450 ppm, respectively). Relative kidney weights were significantly increased in the two highest dose groups of male and female rats exposed to HCBD (21-28% increase in males, 7-22% increase in females). Absolute kidney weights were not reported. Histopathology findings demonstrated dose-related kidney lesions occurring in all exposed animals. These lesions were described as degeneration of the tubule epithelial cells, especially in the straight limbs of the proximal tubules located in the outer zone of the medulla. The LOAEL for the 2-week dietary study was 8 mg/kg-day (50 ppm). A NOAEL value was not identified.

In the reproduction study, female Wistar rats (six females/group) were exposed to 0, 150 or 1500 ppm for 18 weeks (4 weeks prior to mating and a 3-week mating period with untreated males). The number of pups/litter and the pup body weights were measured at partuition and the pups were culled to eight per litter. Offspring body weights were measured at PND 10 and 20 and necropsy of the adult females was conducted at 18 weeks. Organ weights were recorded for the heart, liver, kidneys, spleen, brain, adrenals, thymus and thyroid. Histopathology was performed for these organs and the lungs, pancreas, digestive tract (six segments), urinary bladder, axillary and mesenteric lymph nodes, trachea, spinal cord, and femoral nerve. No conception occurred for female rats exposed to a dietary concentration of 1500 ppm HCBD. Progressive weight loss was seen in rats from this group and an unsteady gait, hind limb weakness and ataxia occurred by 6 weeks of exposure to HCBD. Necropsies were performed during week 10, due to the moribund condition of the animals. Gross examination revealed large

pale kidneys and extensive tubule degeneration was seen by histopathology. Proliferation of bile duct epithelial cells in the liver and fragmentation and demyelination of single fibers of the femoral nerve were also seen.

Five out of six rats in the 150 ppm dose group were fertile with a mean litter size similar to control rats. The birth weight of pups from this treatment group was lower than controls (16% decrease) and a decreased pup body weight was also observed at weaning (19% decrease). The resorption quotient was low for both control and treated rats and no gross malformations of offspring were observed. At 18 weeks, the body weight of treated dams was 15% lower than control dams (231 g for controls, 196 g for 150 ppm group). The average daily dose for the 150 ppm group was estimated to be 11 mg/kg-day, assuming a body weight of 0.196 g and a food consumption rate of 0.015 kg/day (calculated using equations in U.S. EPA, 1988). Relative kidney weight was increased by 22%, as compared with controls. Absolute kidney weights were not reported. HCBD treatment caused histopathological changes in the kidney of dams, including hypercellularity of tubule epithelial cells and hydropic necrosis of cells in the straight limbs of the proximal tubules. Treatment related effects were not observed in other organs or tissues. A LOAEL of 11 mg/kg-day (150 ppm) was derived for the reproduction study, based on maternal effects (decreased weight gain, increased kidney weights and altered kidney histopathology) and decreased fetal body weight.

In the 13-week subchronic study, 60 rats/sex/group received 0, 0.4, 1.0, 2.5, 6.3 or 15.6 mg/kg-day HCBD in arachid oil for 13 weeks. Blood samples were collected at 8 weeks (six rats/sex/group) and analyzed for hemoglobin, hematocrit, RBC count, and total and differential leukocytes. Blood samples were also obtained at study termination and tested for total protein, albumin, globulin, BUN, AST, AP and γ-glutamyl transferase activities. At 10 weeks, urine samples were collected from six rats/sex/group during the 2^{nd}-6^{th} and 7^{th}-21^{st} hour deprivation period of food and water. Urine samples were analyzed for glucose, protein, hemoglobin, ketones and pH. Urine volume and osmolarity were used as measures of the concentrating ability of the kidney. At termination, organs were weighed (heart, liver, kidney, spleen, brain, adrenals, thymus, thyroid and gonads) and a gross pathological examination was carried out on all animals. Key organs and tissues from the control and high-dose groups were processed for histopathological examination (heart, liver, kidneys, spleen, brain, adrenals, thymus, thyroid, lungs, pancreas, six sections of digestive tract, urinary bladder, axillary and mesenteric lymph nodes, trachea, spinal cord, femoral nerve, prostate, skeletal muscle, aorta, Harder's gland, skin, sternum and bone marrow). The HCBD content of kidney, liver and fat samples from high-dose female rats was measured by gas chromatography (GC) analysis.

Body weight gain and food consumption were significantly reduced at the two highest doses in male and female rats, as compared to controls (13-30% decrease in body weight at 6.3 mg/kg-day; >40% reduction in body weight at 15.6 mg/kg-day). No clinical chemistry changes were observed at any dose levels. Following a 21-hour deprivation period, a dose-related decrease in urine osmolarity was observed in female rats that were given HCBD at doses greater than 2.5 mg/kg-day. An increase in urine volume was also observed in the two highest dose groups of female rats (6.3 and 15.6 mg/kg-day), indicating an impairment in the urine concentrating ability of the kidney. No change in urine volume was observed in treated male rats, and urine osmolarity was increased only in high-dose males (15.6 mg/kg-day). Relative

kidney weight was significantly increased in all dose groups of male rats (7-31% increase) and in the two highest dose groups of female rats (19 and 32% increase for 6.3 and 15.6 mg/kg-day respectively). The relative liver weight was increased 8-24% in male rats at doses greater than 1 mg/kg-day, but was only increased in the high-dose group in female rats by 11%. In male rats, the relative weights of the brain and the spleen were increased in the 15.6 mg/kg-day dose group by 45 and 18%, respectively. Increases in the relative weight of the brain and spleen were seen at the two highest doses in female rats (21-29% increase for brain, 14-21% increase for spleen). The relative weight of the gonads was increased in male rats (12 and 40% increase at 6.3 and 15.6 mg/kg-day respectively). Absolute organ weights were not reported in the study.

Though no changes in organ appearance were seen on gross pathological examination, marked histopathological lesions were evident in the kidney, most notably in the proximal tubule, where increases in hypercellularity, necrosis and the incidence of hyperchromatic nuclei were evident. Epithelial cells in treated rats were described as small, basophilic and finely vacuolated, with large hyperchromatic nuclei. At the highest dose in female rats, changes were seen in both the straight and convoluted portions of the tubules with focal necrosis and a thin or absent epithelial brush border. The changes were similar, but less severe, in females given 6.3 mg/kg-day and were limited to the straight portion of the proximal tubule. The brush border was generally unchanged and few necrotic cells were present in the tubule lumen. Only minor effects were seen in female rats given 2.5 mg/kg-day, although tubule epithelial cells were observed to contain enlarged hyperchromatic nuclei. Kidney effects were less pronounced in male rats, as compared to females. Kidney lesions in male rats given 15.6 mg/kg-day were similar in severity to those seen in female rats given 6.3 mg/kg-day. Liver effects were seen in male rats only at doses greater than 6.3 mg/kg-day and consisted of a basophilic granulation of hepatocytes. The GC analysis of kidney, liver and adipose tissue from high-dose female rats revealed no HCBD accumulation in the liver or kidney and only slight accumulation in the fat. NOAEL and LOAEL values of 1.0 and 2.5 mg/kg-day, respectively, were derived from this study based on kidney toxicity in female rats.

Kociba et al. (1977) — Kociba et al. (1977) administered HCBD (99% purity) mixed in feed to 39-40 Sprague-Dawley rats/sex/group for 2 years. Ninety rats of each sex were used as controls. Rats were observed frequently (not quantified) for clinical signs of toxicity. Feed consumption and body weights were monitored in 15 rats/sex/group weekly for the first 3 months of the study, and then for 1 week out of each month until study termination. The average doses for either sex were calculated by the authors to be 0, 0.2, 2 or 20 mg/kg-day. Subsets of animals (5-6/sex/group) were sampled for blood and urine after approximately 12, 22 (males only) or 24 (females only) months. The hematological parameters evaluated included packed cell volume (PCV), RBC count, hemoglobin concentration, total WBC count and differential WBC count. The urinary parameters evaluated were specific gravity, pH and the presence or absence of glucose, protein, ketones, bilirubin and occult blood. Urinary creatinine, coproporphyrin and uroporphyrin were also determined from a urine sample collected over 24 hours. After 1 year, blood samples were collected from an additional subset of animals (five/sex from the high-dose and control groups only) for clinical chemistry determinations. Serum samples were also collected from all rats necropsied at the end of the study. Serum chemistry parameters included blood urea nitrogen (BUN), and AP and ALT activity. All rats (moribund and terminal sacrifice) were necropsied and pieces of all major organs and lesions were excised

and preserved. For the rats that were killed during the course of the study, histopathological evaluation was performed for the liver, kidney, stomach and all tumors or gross lesions. For those sacrificed at term, a fully comprehensive list of organs and tissues was examined microscopically for 10 females at each dose level, 10 males from the 0 and 2 mg/kg-day dose levels and 3 males at the 20 mg/kg-day dose level that survived to term. Histopathology evaluation for the remaining rats that were killed at study termination (including all male rats from the 0.2 mg/kg-day group) was limited to the kidneys, liver, stomach and any gross lesions observed during necropsy.

There was a reduction in body weight gain at the high dose level in both male and female Sprague-Dawley rats that appeared not to be associated with the sporadic changes in food consumption. This decrease in body weight was evident by 27 days in female rats and 69 days in male rats and body weight remained low throughout most of the study. A significant increase in mortality (approximately 20%, estimated from graph) occurred during the last 2 months of the study in male rats that ingested 20 mg/kg-day HCBD. Survival was not reduced in any other HCBD treatment group.

Compound-related changes in hematological parameters were limited to a 20% decrease in RBCs after 22 months in high dose male rats. Routine urinalysis parameters were not affected by HCBD treatment; however, the excretion of coproporphyrins was increased in high-dose male rats at 1 year, mid-dose female rats at 14 months and high-dose female rats at 2 years. No dose response or temporal trend was apparent from these data (see Table 2). A 57% decrease in the excretion of uroporphyrin was also seen in high-dose female rats after 2 years. Clinical chemistry parameters were generally not altered by HCBD treatment for 12 months or 2 years, with the exception of a decrease in ALT activity in high-dose (20 mg/kg-day) males at 12 months and low- and high-dose females (0.2 and 20 mg/kg-day, respectively) at 2 years. This finding in female rats was considered to result from an abnormally increased ALT activity in female control rats and was not considered to be treatment-related.

Table 2. Average Amounts of Coproporphyrins in Urine of Sprague-Dawley Rats in Response to HCBD in Feed (µg/24 hours) (Kociba et al., 1977)

HCBD in feed (mg/kg-day)	1 Year		14 Months		2 Years	
	Male	Female	Male	Female	Male	Female
0	10.2±8.5	5.0±1.3	13.1±3.0	5.6±2.4	6.8±1.8	4.5±2.4
0.2	14.2±2.6	4.7±2.1	13.0±3.8	6.2±3.3	7.1±2.3	5.4±0.8
2.0	18.8±2.4	8.9±5.2	18.3±4.0	10.6[a]±2.4	10.7±2.4	5.8±1.3
20.0	23.1[a]±11.8	9.4±3.5	17.7±12.5	8.4±2.5	14.0 ±9.5	12.3[a]±2.9

Values are means ±SD (n=5).
[a]$p<0.05$ as determined by ANOVA and Dunnett's test.

An increase in the absolute and relative weight of the kidneys was observed in male rats given 20 mg/kg-day HCBD for 22 months. An increase was also observed in relative, but not

absolute, testes weight; however, this may have been due to the observed decrease in body weight. A decrease in the absolute weight of the heart and liver and an increase in the relative weight of the brain and kidney were seen in high-dose female rats. Organ weights in the low- and mid-dose groups of male and female rats were similar to control. Histopathological examination revealed treatment-related kidney lesions in male and female rats consisting of tubular epithelial hyperplasia and proliferation, observed in the mid- and high-dose groups (2 and 20 mg/kg-day) (incidence data not provided) and tubular adenomas and adenocarcinomas in high-dose rats only. The histopathology findings in low-dose rats were similar to controls. The incidence of combined adenomas and carcinomas in kidney was 1/90, 0/40, 0/40 and 9/39 in males and 0/90, 0/40, 0/40 and 6/40 in females, for the control, low-, mid- and high-dose groups, respectively. Metastasis to the lung was noted in two cases. NOAEL and LOAEL values of 0.2 and 2 mg/kg-day, respectively, were derived from this study based on kidney lesions (tubular epithelial hyperplasia and proliferation) observed in male and female rats that ingested HCBD in the diet for 2 years.

Schwetz et al., 1977 — The same research group carried out a combined subchronic and reproductive study (Schwetz et al., 1977) in parallel to that of Kociba et al. (1977). Male and female Sprague-Dawley rats (10-12 males/treatment group, 17 male controls, 20-24 females/treatment group, 34 female controls) received 0, 0.2, 2.0 or 20 mg/kg-day HCBD (99% purity) in feed for 90 days prior to mating, throughout a 15-day mating period, and then through gestation and lactation. Blood and urine samples were collected from control and high-dose rats prior to the end of the study. At study termination, blood samples were taken from the dams prior to necropsy to measure levels of BUN, serum creatinine and ALT activity. The brain, heart, liver, kidneys and testes (males) were obtained from 10 adult rats/sex/group and organ weights were determined. For the controls and high-dose groups, many organs and tissues were excised, weighed and processed for histopathological examination (brain, heart, liver, kidneys, testes, eye, pituitary, thyroid gland, parathyroid gland, trachea, esophagus, lungs, aorta, stomach, pancreas, small intestine, colon, mesenteric lymph nodes, muscle, sciatic nerve, spinal cord, sternum, sternal bone marrow and adrenal gland). Histopathology was also carried out on kidney tissue excised from five animals from each exposure group. Standard indices of reproductive performance were evaluated, and weanling skeletons were examined after alcohol fixation and appropriate extraction and staining. Bone marrow was taken from four adults and four weanlings/sex/group for cytological examination.

No clinical signs were evident in any of the adults receiving HCBD. A decrease in food consumption was noted in high-dose male and female rats. Female rats from this group weighed significantly less than controls throughout the study (22% decrease in final body weight), while male body weights were sometimes, but not always, lower than controls (10% decrease in final body weight). There were no differences among the groups in any reproductive or survival parameters for the dams and neonates (percent pregnant, litter size, gestation survival index, sex ratio, duration of gestation); however, the mean weight of high-dose neonates was significantly reduced (13% decrease) in the 20 mg/kg-day group compared with controls at weaning (21 days of age). No gross abnormalities were observed in neonates at necropsy. Skeletal alterations were not evident in neonates at any dose level.

Among clinical chemistry parameters, BUN was decreased in male rats by 17 and 13% in the 0.2 and 2 mg/kg-day dose groups, respectively, but was similar to controls in the 20 mg/kg-day dose groups. Serum levels of creatinine and ALT did not differ from those of controls. Hematology and urinalysis results were not presented or discussed. A significant increase in the relative weights of the liver (male only, 26% increase) and kidney (27 and 19% increase in males and females, respectively) was observed at the highest dose of HCBD. Absolute liver and kidney weights were not different from control values in any treatment group. An increase in relative brain weight (31% increase) and a decrease in relative heart weight (24% decrease) were also observed in female rats from the 20 mg/kg-day dose group. No changes in absolute brain or heart weight were observed. Kidneys from male rats ingesting 2 or 20 mg/kg-day HCBD were described as roughened with a mottled cortex. No gross abnormalities were noted for female rat kidneys. Histopathological examination revealed renal tubular dilation and hypertrophy with foci of tubular epithelial degeneration and regeneration. The incidence of these kidney lesions in rats ingesting 0, 0.2, 2 or 20 mg/kg-day was 1/5, 0/5, 0/5 and 3/5 for male rats and 0/5, 0/5, 1/5 and 5/5 for female rats. No histopathological kidney lesions were evident in weanling rats. Although these findings are limited by the small number of animals examined for histopathological evaluation, NOAEL and LOAEL values of 0.2 and 2 mg/kg-day, respectively, were derived from this study, based on gross and microscopic kidney lesions in adults rats exposed to HCBD for 90 days prior to mating, 15 days during mating, and throughout gestation and lactation.

Kociba et al., 1971 - HCBD (99% pure) was administered to female Sprague-Dawley rats (4/group) in the diet for 30 days at doses of 0, 1, 3, 10, 30, 65, 100 mg/kg-day (Kociba et al., 1971). Rats were observed daily and feed consumption and body weight gain were recorded weekly throughout the study. Blood samples obtained during necropsy were analyzed for hematology parameters and ALT activity. Organ weights of heart, liver, kidney, spleen and brain were recorded and several organs and tissues were prepared for histopathology evaluation (heart, liver, kidney, spleen, brain, pituitary, thyroid, parathyroid, lung, adrenal, mesenteric lymph node, ovary uterus, stomach and intestinal tract). Clinical signs of toxicity were not observed during the study. The food consumption rate was significantly decreased in rats receiving HCBD doses greater than 30 mg/kg-day. The mean body weight values measured at 28 days were 4, 10, 22 and 28% lower than controls for the 10, 30, 65 and 100 mg/kg-day groups, respectively. A decrease in absolute organ weight was seen in the liver, heart and spleen of rats in the 65 and 100 mg/kg-day dose groups. Absolute kidney weight was increased at 3 mg/kg-day, but was similar to controls for all other treatment groups. An increase in relative organ weight (organ:body weight ratio) was seen in the brain, liver and kidneys of rats given 30, 65 or 100 mg/kg-day HCBD. Hematology results were considered to be within a normal range. No change is AST activity was observed. Gross findings revealed a depletion of abdominal fat deposits in rats given 65 or 100 mg/kg-day HCBD. Histopathology results showed hepatocellular swelling in rats given 100 mg/kg-day HCBD only (4/4 rats). Kidney lesions included tubular epithelial cell degeneration, single cell necrosis and regeneration in all rats (4/4) from the 30, 65 and 100 mg/kg-day dose groups. Liver and kidney lesions were not observed in control rats or in rats given 1 or 3 mg/kg-day (0/4 per group). NOAEL and LOAEL values of 10 and 30 mg/kg-day, respectively, were derived from this study based on renal lesions observed in female Sprague-Dawley rats.

Inhalation Exposure. Few studies were located regarding the toxicity of HCBD by inhalation exposure in animals. Saillenfait et al. (1989) conducted a developmental toxicity study in which groups of 24-25 pregnant Sprague-Dawley rats inhaled 0, 2, 5, 10 or 15 ppm of HCBD for 6 hours/day on days 6-20 of gestation. The pregnant rats were weighed prior to exposure on days 0 and 6 of gestation, and again prior to sacrifice on day 21 of gestation. After sacrifice, the uterus was removed from each female and examined for numbers of implantation and resorption sites and live and dead fetuses. Live fetuses were sexed, weighed and examined for external malformations and cleft palate. Half of the viable fetuses from each litter were examined for soft tissue alterations and the other half were examined for skeletal alterations. No deaths or changes in general behavior were noted for exposed females. There was a concentration-related reduction in maternal weight gain in animals exposed to HCBD. Weight gain was reduced by 8% at 2 ppm, 15% at 5 ppm, 12% at 10 ppm and 39% at 15 ppm. The difference from controls was statistically significant in the 5 and 15 ppm groups.

Mean numbers of implantations, total fetal loss, resorptions and live fetuses were similar in treated and control animals (Saillenfait et al., 1989). Incidence of pregnancy and fetal sex ratio were also unchanged by HCBD exposure. However, body weight of both male and female fetuses was significantly reduced in the 15 ppm group (decreased by 9.5 and 12.5% in males and females, respectively). External examination of fetuses did not find any abnormalities, and no major anomalies were found after skeletal and soft tissue examination. The only minor anomalies were a non-significant incidence of hydroureter at 15 ppm and a non-significant increase in the incidence of extra 14^{th} ribs at 10 ppm. Although there was a significant reduction in fetal weight at the greatest exposure to HCBD, there was no significant retardation of development (e.g., delayed ossification) and the change was accompanied by a reduction in maternal weight gain. This study identified a NOAEL of 2 ppm and a LOAEL of 5 ppm for maternal toxicity (decreased weight gain), and a NOAEL of 10 ppm and a LOAEL of 15 ppm for developmental effects (decreased fetal body weight).

Dow Chemical Company conducted a subchronic inhalation study of HCBD that was described by Torkelson and Rowe (1982), as follows: “small groups of rats, rabbits and guinea pigs exposed 7 hours/day, 100 times to 3 ppm in a 143 day period were adversely affected, but those exposed 129 times in 184 days to 1 ppm were not. The livers and kidneys of the animals exposed to 3 ppm were the organs most affected.” No further details of this study were located. Representatives from Dow have stated that a more detailed report of this study, which was conducted in the 1950s, is no longer available (Dow, 1992).

Respiratory irritation and renal effects were observed in short-term, repeated inhalation studies of HCBD in rats. Alderley Park SPF rats (four rats of each sex for each treatment) were exposed to concentrations of HCBD ranging from 5 to 250 ppm for durations up to 3 weeks (Gage, 1970). A day after exposure was terminated, animals were sacrificed and necropsied. The following organs were routinely examined microscopically for damage: lungs, liver, kidneys, spleen and adrenals. Blood and urine tests were normal for all treatments. At 250 ppm of HCBD (2 x 4 hours), irritation and breathing difficulties were observed (more pronounced in females). Necropsy showed degeneration of the middle renal proximal tubules and the adrenal cortex. At 100 ppm (6 hours/day, 5 days/week, 12 days) irritation and respiratory difficulties were observed; animals had poor condition and weight loss. Females had slight anemia and two

died. Necropsy showed enlarged adrenal glands and pale, enlarged kidneys with degeneration of the renal cortical tubules and epithelial regeneration. At 25 ppm (6 hours/day, 5 days/week, 3 weeks) respiratory difficulties and poor condition were observed. Females had diminished weight gain. At necropsy, kidneys were pale and enlarged with damage to the renal proximal tubules. Ten ppm (6 hours/day, 5 days/week, 3 weeks) produced diminished weight gain in females, but no organ damage. Exposure to 5 ppm (6 hours/day, 5 days/week, 3 weeks) caused no symptoms of toxicity or organ damage.

DeCeaurriz et al. (1988) assessed respiratory irritation and kidney damage after acute exposure of male Swiss OF_1 mice to HCBD. The respiratory rates of mice (six mice per treatment group) were measured during a 15-minute oronasal exposure to HCBD (83, 143, 155, 210 or 246 ppm) using individual body plethysmographs. The decreases in respiratory rate recorded for each concentration were used to calculate the concentration associated with a 50% decrease in respiratory rate (RD_{50}). The RD_{50} for HCBD was 211 ppm. In a previous study, DeCeaurriz et al. (1981) calculated the RD_{50} for a number of different chemicals. The RD_{50} for hexachlorobutadiene places it among the more potent irritants. For instance, the RD_{50} for phenol was 166 ppm and that of formaldehyde was 5.3 ppm, while the RD_{50} of toluene was 3373 ppm and that of xylene was 1467 ppm (DeCeaurriz et al., 1981).

Mice were also exposed to various concentrations of HCBD (2.75, 5, 10 and 25 ppm) or clean filtered air for 4 hours (DeCeaurriz et al., 1988). After a recovery period of 24 hours, the animals were sacrificed and their kidneys were examined microscopically for damaged tubules and alkaline phosphatase staining. There was a significant, concentration-related increase in nephrotoxicity associated with HCBD exposure. The percentage of altered renal tubular cross-sections increased from 4% in the 2.75 ppm group to 92% in the 25 ppm group (versus 0.2-1.5% in the corresponding control groups). The researchers estimated an EC_{50} of 7.2 ppm for kidney histopathology produced by HCBD. On the basis of these findings, the researchers concluded that the kidney is a more sensitive target for HCBD than the respiratory tract following acute inhalation exposure in the mouse. However, DeCeaurriz et al. (1988) did not perform a histopathological examination of the upper respiratory tract. A more recent evaluation of the applicability of sensory irritation tests (Bos et al., 1992) has described a number of compounds for which histopathological damage was observed at exposure levels more than 10 times lower than the RD_{50}.

Although respiratory tract effects of HCBD have not been studied following chronic exposure, it is reasonable to suspect that such effects may be important for this chemical. Nasal toxicity was a prominent finding in chronic bioassays of the structurally related chemical 2-chloro-1,3-butadiene (chloroprene) in rats and mice (NTP, 1998).

Other Studies

The mode of action for the kidney toxicity of HCBD has been described (reviewed in Green et al., 2003; NTP, 1991; Dekant et al., 1990). HCBD is metabolized in the liver to a glutathione conjugate, which is further transformed by γ-glutamyl transpeptidase and dipeptidase enzymes to yield a cysteine conjugate. The cysteine conjugate may be cleaved by the renal β-lyase enzymes to give toxic thiol intermediates that cause localized kidney damage. The

cysteine conjugate may also be metabolized by N-acetyl transferase to form a N-acetyl cysteine conjugate that can be excreted in the urine or converted back to the cysteine conjugate by acylase enzymes. The nephrotoxicity of HCBD is linked to the relative activity of the renal β-lyase enzyme and the amount of cysteine conjugate available to be metabolized to toxic intermediates.

Green et al. (2003) compared the key metabolic steps for HCBD in both rat and human tissues. Human liver and kidney samples were obtained as excess tissue during organ transplantation. In vitro studies were used to evaluate glutathione conjugation of HCBD (in liver microsomes), the metabolism of cysteine conjugates by renal β-lyase (in kidney cytosol and mitochondria) or N-acetyl transferase (kidney microsomes) and the metabolism of the N-acetyl cysteine conjugate by acylase enzymes (kidney cytosol). The metabolic rates (Vmax) for each of these steps were lower in humans as compared to rats (5-fold lower for glutathione conjugation, 3-fold lower for β-lyase activity and 3.5-fold lower for N-acetyl transferase activity). Acylase enzyme activity was not detected in human kidney cytosol. The metabolic rate constants obtained for rats and humans were used in a physiologically-based pharmacokinetic (PBPK) model to quantify metabolism through the β-lyase pathway to form reactive intermediates. The uptake and distribution of HCBD was estimated in the PBPK model using measured partition coefficients and standard values for physiological parameters. The PBPK model predicted that metabolism by the β-lyase pathway is approximately 20-fold lower in humans than in rats exposed to the same inhalation concentration. The predicted decrease in the formation of β-lyase metabolites was related to decreased uptake of HCBD, lower glutathione transferase and β-lyase activities and the absence of acylase activity in human kidney. Comparable model predictions for the oral exposure route were not provided in this study.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR HCBD

No data were located regarding the oral toxicity of HCBD in humans. Kidney toxicity was the primary effect of oral HCBD exposure in laboratory animals. Short-term studies (2-4 weeks in duration) demonstrated necrosis and degeneration of kidney tubules at high doses (>10 mg/kg-day) (Jonker et al., 1993; NTP, 1991; Yang et al., 1989; Harleman and Seinan, 1979; Kociba et al., 1971). Necrosis was less severe at lower doses and regeneration of kidney tubules was observed. In subchronic and chronic studies, the primary histopathological change observed was renal tubule regeneration, also characterized as hyperplasia and proliferation, and necrosis was not generally seen (NTP, 1991; Yang et al., 1989; Harleman and Seinen, 1979; Kociba et al., 1977, Schwetz et al., 1977). Kidney effects were most prevalent in the straight limbs of the proximal tubule in the outer zone of the medulla; however, the convoluted portions of the proximal tubule were also involved at high doses (Harleman and Seinen, 1979). The regenerative hyperplasia observed in tubule epithelial cells (NTP, 1991; Yang et al., 1989; Harleman and Seinen, 1979; Kociba et al., 1971) may be a response to HCBD-induced cell injury and/or may be a precursor to the renal neoplasms that were observed following chronic exposure (Kociba et al., 1977). The chronic and subchronic oral toxicity studies for HCBD are summarized in Table 3.

Table 3. Chronic and Subchronic Oral Toxicity Studies for HCBD

Species	Dose/Duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
B6C3F$_1$ mice	13-week dietary study; 0, 0.1, 0.4, 1.5, 4.9 or 16.8 mg/kg-day in males; 0, 0.2, 0.5, 1.8, 4.5 or 19.2 mg/kg/day in females	0.2	0.5	Renal tubule regeneration	NTP (1991); Yang et al. (1989)
Wistar rats	13-week oral gavage study; 0, 0.4, 1.0, 2.5, 6.3 or 15.6 mg/kg-day HCBD in arachid oil	1.0	2.5	Tubule epithelial cell with enlarged hyperchromatic nuclei; focal necrosis at higher doses	Harleman and Seinen (1979)
Sprague-Dawley rats	2-year dietary study; 0, 0.2, 2 or 20 mg/kg-day	0.2	2	Renal tubule hyperplasia and proliferation	Kociba et al. (1977)
Sprague-Dawley rats	Dietary study, 13 weeks premating, 15 day mating period and throughout gestation and lactation; 0, 0.2, 2 or 20 mg/kg-day	0.2	2	Renal tubule degeneration and regeneration	Schwetz et al. (1977)

The quantal Benchmark Dose (BMD) models in the BMD software package (U.S. EPA, 2007; Version 1.3.2) were fit to the female mouse renal tubule regeneration data in Table 1 (NTP, 1991; Yang et al., 1989). The gamma, log-probit, Weibull and log-logistic give virtually the same fit with indistinguishable Akaike Information Criterion (AIC). The BMD and BMDL10 values were all the same at 0.2 and 0.1 mg/kg-day, respectively. The Weibull had the best fit in the region of the BMR (lowest absolute scaled residual at 0.2 mg/kg-day, although the differences among the models are minimal (see Appendix 1). The 1st-order multistage fit adequately ($p = 0.37$) but had a much higher AIC than the aforementioned model fits. Therefore, the point of departure (POD) is set equal to the common BMDL10 of 0.1 mg/kg-day.

The **subchronic p-RfD of 1E-3 mg/kg-day** is based on the $BMDL_{10}$ of 0.1 mg/kg-day for renal tubule regeneration observed in a 13-week dietary study in mice (NTP, 1991; Yang et al., 1989). Kidney toxicity was also seen in a 13-week gavage study in rats at higher doses (Harleman and Seinen, 1979).

The subchronic p-RFD is derived by dividing the $BMDL_{10}$ of 0.1 mg/kg-day by a composite UF of 100, as follows:

Subchronic p-RfD	=	NOAEL/ UF
	=	0.1 mg/kg-day / 100
	=	**0.001 or 1E-3 mg/kg-day**

The composite UF of 100 includes factors of 3 ($10^{0.5}$) each for animal-to-human extrapolation and database deficiencies, and a factor of 10 for interindividual variability.

The interspecies UF of 3 was used to account for pharmacodynamic differences across species. The role of metabolism in HCBD-induced kidney toxicity is well established. Pharmacokinetic differences between the rat and the human were investigated by Green et al. (2003). In vitro studies were used to evaluate key steps in the metabolism of HCBD (glutathione conjugation in the liver, β-lyase, N-acetyl transferase and acylase enzyme activity in the kidney). The metabolic rate for each of these steps was lower in humans as compared to rats, suggesting that humans may be less sensitive to the kidney toxicity of HCBD (no pharmacokinetic adjustment was necessary). Although comparable in vitro metabolism data are not available for mice, the dose response data suggest that rats and mice are similarly sensitive to the kidney toxicity caused by HCBD. Given the large difference (20-fold) in predicted toxic metabolite formation in the PBPK model (Green et al., 2003), there is marginal justification for reducing UF_A to unity, despite the lack of information on toxicodynamic differences between rats (or mice) and humans. However, a somewhat limiting assumption is already made about the similarity of mice and rats for the metabolism of HCBD and there is no information on the relative *in vivo* abundance of key enzymes across species, only on specific activities. These limitations preclude further reduction of UF_A. The interspecies UF of 3 was therefore considered appropriate for both rats and mice.

The interindividual variability UF of 10 is used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status or genetic makeup might vary in the disposition of, or response to, HCBD. A partial UF of 3 for database deficiencies is selected due to the lack of a multigeneration reproductive toxicity study. Prenatal and postnatal developmental toxicity studies are available for HCBD using the oral and inhalation exposure route (Field et al., 1990; Harleman and Seinen, 1979; Saillenfait et al., 1989). There was little assessment of the immune and nervous systems in the literature. With respect to the latter, Harleman and Seinen (1979) demonstrated neuropathy in rats at 1500 ppm (150 mg/kg-day) after 18 weeks of exposure. As this LOAEL is more than 3 orders-of-magnitude greater than the BMDL of 0.1 mg/kg-day, it is probably not much of a concern, either for the subchronic p-RfD or for longer periods of exposure relative to the chronic p-RfD.

Confidence in the critical study is medium. NTP (1991)/Yang et al. (1989) was a well-conducted, 13-week dietary study with relatively small number of animals (10/group). The critical effect (kidney lesions) was well studied. Limitations include lack of hematology and clinical chemistry and lack of histopathology on organs other than the kidney. NOAEL and LOAEL values were derived from the study based on kidney toxicity. Confidence in the database is medium. An additional subchronic oral gavage study showed similar effects at doses that were approximately 5-fold higher (Harleman and Seinen, 1979). Limitations of the database

include the lack of multigeneration reproductive toxicity data. Prenatal and postnatal developmental toxicity studies have been performed (Field et al., 1990; Harleman and Seinen, 1979; Saillenfait et al., 1989). Overall, confidence in the subchronic p-RfD is medium.

The **chronic p-RfD of 1E-3 mg/kg-day** is also based on renal tubule regeneration in the 13-week dietary study in mice (NTP, 1991; Yang et al., 1989), as the BMDL of 0.1 mg/kg-day is lower than the NOAEL of 0.2 mg/kg-day for renal effects in the 2-year rat study (Kociba et al., 1977). The incidence of kidney lesions was not reported for each dose group in the 2-year study. Therefore, benchmark dose modeling could not be used to derive a point of departure. Therefore, the chronic p-RfD is derived by dividing the subchronic $BMDL_{10}$ of 0.1 mg/kg-day by a composite UF of 100, as follows:

Chronic p-RfD	=	NOAEL / UF
	=	0.1 mg/kg-day / 100
	=	**0.001 or 1E-3 mg/kg-day**

The composite UF of 100 includes factors of 3 ($10^{0.5}$) each for animal-to-human extrapolation and database deficiencies, and a factor of 10 for interindividual variability. A subchronic-to-chronic uncertainty factor is not required because the chronic 2-year rat study indicates that prolonged exposure does not result in toxicity at lower doses than for subchronic exposure. The interspecies UF of 3 was used to account for pharmacodynamic differences across species as described previously for the subchronic p-RfD. The interindividual variability UF of 10 is used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status or genetic makeup might vary in the disposition of, or response to, HCBD. A partial UF of 3 for database deficiencies is selected due to the lack of a multigeneration reproductive toxicity study. Prenatal and postnatal developmental toxicity studies are available for HCBD using the oral and inhalation exposure route (Field et al., 1990; Harleman and Seinen, 1979; Saillenfait et al., 1989).

Overall confidence in the chronic p-RfD is medium for the same reasons as for the subchronic p-RfD.

FEASIBILITY OF DERIVING PROVISIONAL CHRONIC AND SUBCHRONIC RfCs FOR HCBD

The available data are inadequate to support derivation of a provisional inhalation RfC for HCBD. Reduced body weight gain was observed in dams following inhalation exposure in the Saillenfait et al. (1989) rat developmental toxicity study (NOAEL of 2 ppm). However, this study included limited evaluation of non-developmental endpoints, no examination of the respiratory tract and no assessment of kidney toxicity, the critical effort for oral exposure. The only other inhalation study of appropriate duration to consider for RfC derivation is the Dow Chemical study briefly described by Torkelson and Rowe (1982). However, the existing description of this study provides insufficient information to assess the study, and attempts to obtain more detailed information about the study were unsuccessful.

The database for oral toxicity of HCBD is more extensive than that for inhalation toxicity (ATSDR, 1994). The kidney appears to be the most sensitive target of HCBD by oral exposure. However, due to overt signs of respiratory irritation and uncertainty regarding the relative sensitivity of the respiratory tract as compared to the kidney with long-term inhalation exposure, an RfC is not derived.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR HEXACHLOROBUTADIENE

The carcinogenicity assessment, which includes an oral slope factor and inhalation unit risk, is on IRIS (U.S. EPA, 1991b).

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APPENDIX

BENCHMARK DOSE MODELING RESULTS (BMDS, VERSION 1.3.2): FEMALE MOUSE RENAL TUBULE REGENERATION DATA (NTP, 1991; Yang et al., 1989; see Table 1 in main document).

BMDS MODEL RUN: Weibull

~~~~~~

The form of the probability function is:

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

Power parameter is restricted as power >=1

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0454545
Slope = 0.158569
Power = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	Slope	Power
Slope	1	0.93
Power	0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	23.743	24.862
Power	3.36618	1.18082
~~~~~~

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-6.50166			
Fitted model	-6.50166	1.54667e-011	4	1
Reduced model	-38.1909	63.3784	5	<.0001

AIC: 17.0033

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0	10	0
0.2000	0.1000	1.000	1	10	-3.148e-006
0.5000	0.9000	9.000	9	10	2.358e-006
1.8000	1.0000	10.000	10	10	0
4.5000	1.0000	10.000	10	10	0
19.2000	1.0000	10.000	10	10	0

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.2

BMDL = 0.0992532

BMDS MODEL RUN: gamma

~~~~~~

The form of the probability function is:

P[response]= background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function

Power parameter is restricted as power >=1

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial (and Specified) Parameter Values
Background = 0.0454545
Slope = 2.65597
Power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates

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

	Slope	Power
Slope	1	0.98
Power	0.98	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Slope	24.0026	14.4638
Power	8.1874	4.77621

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-6.50166			
Fitted model	-6.50166	1.40514e-009	4	1
Reduced model	-38.1909	63.3784	5	<.0001

AIC: 17.0033

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0	10	0
0.2000	0.1000	1.000	1	10	3.123e-005
0.5000	0.9000	9.000	9	10	1.059e-005
1.8000	1.0000	10.000	10	10	1.26e-005
4.5000	1.0000	10.000	10	10	0
19.2000	1.0000	10.000	10	10	0

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.200001

BMDL = 0.110583

BMDS MODEL RUN: log-logistic

~~~~~~

The form of the probability function is:

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

Slope parameter is restricted as slope >= 1

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 1.20641
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	0.93
slope	0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	5.53548	2.00046
slope	4.8069	1.61467

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-6.50166			
Fitted model	-6.50405	0.00478203	4	1
Reduced model	-38.1909	63.3784	5	<.0001

AIC: 17.0081

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0	10	0
0.2000	0.0997	0.997	1	10	0.003517
0.5000	0.9006	9.006	9	10	-0.006035
1.8000	0.9998	9.998	10	10	0.04836
4.5000	1.0000	10.000	10	10	0.005346
19.2000	1.0000	10.000	10	10	0.0001635

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.200154

BMDL = 0.122758

BMDS MODEL RUN: log-probit

~~~~~~

The form of the probability function is:

P[response] = Background
+ (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

where CumNorm(.) is the cumulative normal distribution function

Slope parameter is restricted as slope >= 1

Total number of observations = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0
intercept = -0.328418
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

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

	intercept	slope
intercept	1	0.93
slope	0.93	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	3.22052	1.03364
slope	2.7973	0.834194

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-6.50166			
Fitted model	-6.50167	1.14638e-005	4	1
Reduced model	-38.1909	63.3784	5	<.0001

AIC: 17.0033

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0000	0.000	0	10	0
0.2000	0.1000	1.000	1	10	2.746e-005
0.5000	0.9000	9.000	9	10	-4.763e-005
1.8000	1.0000	10.000	10	10	0.002394
4.5000	1.0000	10.000	10	10	7.437e-007
19.2000	1.0000	10.000	10	10	0

Chi-square = 0.00 DF = 4 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.200001

BMDL = 0.125663

BMDS MODEL RUN: Multistage (1st-order)

~~~~~~

The form of the probability function is:

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

The parameter betas are restricted to be positive

Total number of observations = 6
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

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

Default Initial Parameter Values
Background = 1
Beta(1) = 4.46022e+018

Asymptotic Correlation Matrix of Parameter Estimates

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

Beta(1)

Beta(1) 1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0	NA
Beta(1)	2.51013	0.870953

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-6.50166			
Fitted model	-9.83272	6.66212	5	0.247
Reduced model	-38.1909	63.3784	5	<.0001

AIC: 21.6654

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0000	0.000	0	10	0.000
i: 2					
0.2000	0.3947	3.947	1	10	-1.234
i: 3					
0.5000	0.7149	7.149	9	10	0.908
i: 4					
1.8000	0.9891	9.891	10	10	1.011
i: 5					
4.5000	1.0000	10.000	10	10	1.000
i: 6					
19.2000	1.0000	10.000	10	10	0.000

Chi-square = 5.43 DF = 5 P-value = 0.3661

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0419741

BMDL = 0.0265053