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Provisional Peer Reviewed Toxicity Values for

Pentachloroethane (CASRN 76-01-7)

Superfund Health Risk Technical Support Center
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Office of Research and Development
U.S. Environmental Protection Agency
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Acronyms and Abbreviations

AIC	Akaike Information Criterion
BMD	benchmark dose
BMDL	95% lower bound on the benchmark dose
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
POD	point of departure
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

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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 assessment for pentachloroethane is available on IRIS (U.S. EPA, 2007a), the HEAST (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). No relevant documents were located in the Chemical Assessment and Related Activities (CARA) list (U.S. EPA, 1991b, 1994). ATSDR (2007), and WHO (2007) have not produced documents regarding pentachloroethane. IARC (1999a) concluded that pentachloroethane is not classifiable as to its carcinogenicity in humans (Group 3). Comprehensive literature searches were conducted in 2006 of the following databases: TOXLINE (1965-2006), CANCERLINE (1970-2006), MEDLINE (1966-2006), GENETOX, DART, CCRIS, CHEMID, RTECS, EMIC, ETICBACK and TSCATS for toxicity studies of pentachloroethane.

REVIEW OF PERTINENT LITERATURE

Human Studies

There are no epidemiologic data relevant to the carcinogenicity of pentachloroethane in humans (IARC, 1999a).

Animal Studies

In conjunction with a carcinogenesis bioassay, the National Toxicology Program (NTP, 1983) performed short term (14-day) and subchronic (13-weeks) toxicity studies for pentachloroethane in F344/N rats and B6C3F1 mice. In the 14-day study, groups of 5 F344/N rats/sex/dose and 5 B6C3F1 mice/sex/dose were administered by gavage 10, 50, 100, 500 or 1000 mg/kg pentachloroethane in corn oil for 14 days. Vehicle controls received only corn oil. All animals at the 1000 mg/kg-day dose died. Sixty percent of the animals at the 500 mg/kg-day dose died. The only clinical sign observed was lethargy among the rats at the two highest doses. No gross or microscopic lesions were found in the animals that died. Body weights of the animals that survived at the 500 mg/kg-day dose were slightly reduced compared to controls (10% and 15% in the males and females, respectively). No compound-related gross or microscopic (examined liver, lungs and spleen) changes were noted in any of the treated animals. The dose level of 500 mg/kg-day is identified as an FEL for mortality. Given the lack of histopathological effects and the minimal number of animals, a NOAEL or LOAEL cannot be determined with any confidence.

In the NTP (1983) subchronic study, groups of 10 F344/N rats/sex/dose were administered by gavage 5, 10, 50, 125 or 250 mg/kg pentachloroethane in corn oil for 13 weeks (5 times/week). Groups of 10 B6C3F1 mice/sex/dose received 5, 10, 50, 100 or 500 mg/kg by the same protocol. Vehicle controls received only corn oil. The composition of the material was 89.5% pentachloroethane, 10.4% hexachloroethane and 0.55% tetrachloroethylene. All rats survived the 13 week exposure period, and no compound-related gross or histopathologic effects were observed. All male mice survived to the end of the 13 week study period, and their body weight gains were comparable to controls. One female mouse in the 500 mg/kg dose group died. No compound-related gross or histopathologic effects were observed in either rats or mice. Final body weights were depressed by 5% for male and 9% for female rats, compared to control groups. No statistical analysis was reported by NTP (1983). An analysis of the mean body weights of rats administered pentachloroethane (presented in Table 4 in the NTP, 1983 report) shows reductions in body weight gain of 10% for male rats and 17% for female rats at the 250 mg/kg-day dose level compared to controls. No statistical analyses can be performed on these data, as neither the individual animal data nor standard errors are presented. The final body weight of high dose females was reduced 8% compared with controls. An analysis of mean body weights of mice administered pentachloroethane (presented in Table 8 in the NTP, 1983 report) shows a 29% reduction in body weight gain for female mice at 500 mg/kg-day compared to controls. A 14% reduction in body weight gain was calculated for female mice at both 5 and 100 mg/kg-day dose groups, with the two intermediate dose groups (10 and 50 mg/kg-day) showing no effects on body weight gain (i.e., 100% of controls). Thus, the significance of the 19% reduction at 5 mg/kg-day is questionable. Based on the body weight gain data, a LOAEL of 250 mg/kg-day ($LOAEL_{ADJ} = 180$ mg/kg-day after adjusting for gavage schedule) is suggested for rats and 500 mg/kg-day ($LOAEL_{ADJ} = 357$ mg/kg-day) for mice. The respective NOAELs are 125 mg/kg-day ($NOAEL_{ADJ} = 89$ mg/kg-day) for rats and 100 mg/kg-day ($NOAEL_{ADJ} = 71$ mg/kg-day) for mice.

The pentachloroethane carcinogenesis/general toxicity bioassay part of the NTP (1983) study was also performed in F344/N rats and B6C3F1 mice. This was the only available study

that evaluated the chronic (noncancer) toxicity and carcinogenicity of pentachloroethane in animals. Male and female F344/N rats (50/sex/dose) were administered doses of 75 or 150 mg/kg pentachloroethane for 103 weeks (5 times/week) by gavage. The composition of the material was 95.5% pentachloroethane, 4.2% hexachloroethane and 0.125% trichloroethylene, which differed slightly from the material used in the shorter-term studies (10.4% hexachloroethane and 0.55% tetrachloroethylene impurities). Male rats maintained normal body weights through the first 76 weeks of the study, after which body weights were slightly less than control animals. Female rats maintained normal body weights through the first 42 weeks of the study, after which body weights were decreased compared to control animals. Final mean body weights were decreased 4-5% for treated males compared to controls, and 8-12% for treated females compared to controls. No dose-response relationship was observed for decreased body weight gain, although there was a trend for reduced body weight for all treated animals beginning at week 40 for females and week 70 for males (Figure 1; NTP, 1983). Survival among male rats at the end of the study was 82% for controls, 66% for the low dose group, and 52% for the high dose group. Early mortality was evident for all treated males compared to controls. A sharp downward trend in survival was apparent starting at week 15, with survival down to 88% by week 18 for both treatment groups (Figure 2; NTP, 1983). Male control animals did not experience reduced survival until after week 80. Survival among female rats at the end of the study was 76% for controls, 72% for the low dose group, and 50% for the high dose group. A reduced survival trend was evident for high-dose females, only. In regards to nonneoplastic lesions, chronic inflammation of the kidney (nephropathy) and interstitial inflammation of the lung were observed in male rats with a positive dose-response relationship (Table 1).

Endpoint	Control	75 mg/kg (low dose)	150 mg/kg (high dose)
Nephropathy	4/50 (8%)	14/49 (29%)	33/50 (66%)
Renal papilla mineralization	4/50 (8%)	29/49 (59%)	29/50 (58%)
Lung interstitial inflammation	5/50 (10%)	10/49 (20%)	15/50 (30%)
Acute/chronic lung inflammation	27/50 (54%)	31/49 (63%)	19/50 (38%)

Nephropathy in the male rat was characterized by interstitial fibrosis, interstitial accumulation of mononuclear inflammatory cells, and severe tubular dilation in the inner cortex with some evidence of giant cells and casts. This toxic endpoint is distinct from “aging” nephropathy where interstitial fibrosis and tubular dilation are not as severe; giant cells within tubules are not characteristic of aging nephropathy. Glomerular hyalinization was also observed. Treated rats displayed increased incidences of mineralization of the renal papilla. In the lung, male rats displayed interstitial inflammation. However, an association between lung inflammation and pentachloroethane exposure could not be conclusively established because of the higher incidence of acute/chronic lung inflammation in the control group compared to the high dose group. Female rats did not exhibit any exposure-related nonneoplastic lesions. However, because of the decreased survival of males in both treatment groups, an FEL of 54

mg/kg-day (75 mg/kg-day dose after adjustment for dosing schedule) is established in this study (NTP, 1983).

Table 2 summarizes the noncancer effects observed in the NTP (1983) report.

TABLE 2. Table of Noncancer Effects (NTP, 1983)					
Species	Duration	Dose/Exposure	Endpoint	NOAEL (mg/kg-day)^a	LOAEL (mg/kg-day)^a
Male F344/N rats	13 weeks	5, 10, 50, 125 or 250 mg/kg	Decreased body weight gain	89	180
Female F344/N rats	13 weeks	5, 10, 50, 125 or 250 mg/kg	Decreased body weight gain	89	180
Male B6C3F1 mice	13 weeks	5, 10, 50, 100 or 500 mg/kg	No effects observed	500	None
Female B6C3F1 mice	13 weeks	5, 10, 50, 100 or 500 mg/kg	One mouse in 500 mg/kg group died, body weight decreased	71	357
Male F344/N rats	103 weeks	75 or 150 mg/kg	Nephropathy, reduced survival	None	54 ^b
Female F344/N rats	103 weeks	75 or 150 mg/kg	No effects observed	110	None
Male B6C3F1 mice	103 weeks	250 or 500 mg/kg	Decreased body weight gain, high mortality both dose groups	None	180 (FEL)
Female B6C3F1 mice	103 weeks	250 or 500 mg/kg	Decreased body weight gain, high mortality both dose groups	None	180 (FEL)

^a adjusted for exposure regimen (from experimental gavage dosing for 5 days per week to 7 days per week)

^b also an FEL for reduced survival

The incidences of primary tumors exhibited negative dose-response trends when statistically analyzed. Male rats exhibited negative trends for incidences of subcutaneous tissue fibromas and pituitary adenomas (primarily chromophobe). Female rats exhibited a negative trend for the incidence of pituitary adenomas. The peer-review panel (for NTP, 1983) commented that these negative trends could be explained by the decreased survival by the end of the study. Additionally, after re-examination of the slides, NTP (1983) determined that the incidence of renal tubular-cell adenomas was increased in treated male rats with a dose-related trend ($p < 0.05$). The incidence of this (historically rare) tumor was 0/50, 1/49 and 4/50 for the control, low-dose and high-dose groups, respectively. In addition, kidney adenocarcinomas were observed in one control and one low-dose male rats, with an additional carcinoma of the kidney reported for another low-dose male.

Male and female B6C3F1 mice (50/sex/dose) were administered doses of 250 or 500 mg/kg pentachloroethane for 103 weeks (5 times/week) by gavage. The composition of the material was 95.5% pentachloroethane, 4.2% hexachloroethane and 0.125% trichloroethylene, which differed slightly from the material used in the shorter-term studies (10.4% hexachloroethane and 0.55% tetrachloroethylene impurities). Pentachloroethane exposure resulted in a significant and dose-related adverse effect on survival. For the high dose males, 42/50 (84%) had died by week 41 of the study. The 8 remaining high dose males were killed

during week 41 for histopathologic evaluation. To provide control samples, 25 male control mice were killed in week 44. Of the remaining male control mice, 19/25 (76%) survived to the end of the study. For the low dose males, 22/50 (44%) survived to the end of the study. Of the female control mice, 38/50 (76%) survived to the end of the study. Survival in the low dose female group was 9/50 (18%) at study termination. The high dose females were all dead by week 74. Both exposure levels resulted in significantly decreased body weight gain in both sexes. The high dose males stopped growing appreciably beginning in week 12. By week 52, the low dose males had significantly lower body weights than controls. While male control mice body weights remained constant, low dose males exhibited a mean body weight decrease of 30% between weeks 42 and 104. High dose and low dose females exhibited lower body weights than controls beginning at weeks 26 and 72, respectively. The body weights for high dose females remained constant between weeks 26 and 74, although the last high dose female died at week 74. Body weights of the low dose females were decreased by more than 10% after weeks 75-80.

Due to the high mortality in the male high dose group and the killing of 25 male control mice in week 45, tumor incidences were compared in male mice at 0-52 weeks, 53-103 weeks, and at study termination. Individual time interval comparisons were combined statistically to obtain an overall result. Neoplastic lesions were observed primarily in the liver of mice of both sexes. Incidences of hepatocellular carcinomas and hepatocellular adenomas were significantly increased in exposed female mice compared to control (Table 3). Male mice also exhibited statistically increased incidences of hepatocellular carcinomas, although early mortality of high dose males precluded an evaluation of their lifetime incidence of hepatocellular carcinomas. The combined incidence of hepatocellular adenomas and hepatocellular carcinomas in female mice, with poly-3 adjustment (Bailer and C.J. Portier, 1988), was selected for oral cancer slope factor derivation.

NTP concluded that: "Under the conditions of this bioassay, technical grade pentachloroethane containing 4.2% hexachloroethane (a known carcinogen in mice) was not carcinogenic in F344/N rats. The decreased survival of dosed rats might have reduced the sensitivity for a carcinogenic response in this species. Pentachloroethane was nephrotoxic to male rats. Technical grade pentachloroethane was carcinogenic for B6C3F1 mice, causing hepatocellular carcinomas in males and females, and adenomas in females."

This study was reported in the scientific literature by Mennear et al. (1982). This NTP (1983) study was cited by IARC (1986, 1999) as limited evidence for the carcinogenicity of pentachloroethane in experimental animals. Combined with the lack of epidemiological data for exposure to pentachloroethane, IARC (1999) stated that pentachloroethane was not classifiable as to its carcinogenicity in humans.

Other Studies

NTP (1983) stated in their discussion that NTP mutagenicity tests were negative in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 either in the presence or absence of an exogenous metabolic activation system (S9) from the livers of Aroclor-induced rats or hamsters (NTP unpublished results, as cited in IARC, 1986).

TABLE 3. Incidences of Neoplastic Lesions in Mice			
Endpoint	Control	250 mg/kg (low dose)	500 mg/kg (high dose)
Female Mice			
Hepatocellular carcinomas	1/46 (2%)	28/42 (67%)	13/45 (29%)
Hepatocellular adenomas	2/46 (4%)	8/42 (19%)	19/45 (42%)
Combined tumors (poly-3 adjusted)	3/44 (7%)	36/40 (90%)	32/34 (93%)
Male mice			
Hepatocellular carcinomas			
Overall	4/48 (8%)	26/44 (59%)	7/45 (16%)
0-52 weeks	0/25 (0%)	1/2 (50%)	7/45 (16%)
53-103 weeks	0/4 (0%)	9/18 (50%)	0/0
Terminal kill	4/19 (21%)	16/24 (67%)	0/0
Hepatocellular adenomas			
Overall	10/48 (21%)	4/44 (9%)	7/45 (16%)
0-52 weeks	5/25 (20%)	0/2 (0%)	7/45 (16%)
53-103 weeks	0/4 (0%)	2/18 (11%)	0/0
Terminal kill	5/19 (26%)	2/24 (8%)	0/0

Galloway et al. (1987) reviewed and summarized the data on sister chromatid exchanges (SCE) and chromosome aberrations in Chinese hamster ovary (CHO) cells for 108 chemicals. Pentachloroethane was positive for SCE when no exogenous metabolic system was present, but negative when rat liver S9 was added. Chromosome aberrations were negative both with and without exogenous rat liver S9.

Matsuoka et al. (1996) used a Chinese hamster lung fibroblast cell line (CHO/IU) to evaluate the ability of pentachloroethane to induce chromosomal aberrations. This study used a different cell line and protocol than the negative NTP study. Three different exposure times (6, 24 or 48 hours) were paired with two recovery times (0 or 18 hours). Marginal results for structural chromosomal aberrations were observed for the 24 hour exposure/0 hour recovery protocol and the 6 hour exposure/18 hour recovery; the addition of rat liver S9 resulted in negative results. Pentachloroethane induced polyploidy in 24 hour exposure treatments with a dose-response relationship. However, polyploidy induction was marginal in 48 hour exposure treatments, most likely due to spindle poisons. The overall assessment by the study authors of pentachloroethane's ability to induce chromosomal aberrations was positive.

Sofuni et al. (1996) summarized the results from various laboratories using the mouse lymphoma assay (L5178Y (TK+/TK-)) for the detection of in vitro clastogens and spindle poisons. Pentachloroethane was positive in the absence of a rat liver S9 fraction, but negative when S9 was present.

Nastainczyk et al. (1982) evaluated the metabolism of pentachloroethane using rat liver microsomes from male Sprague-Dawley rats pretreated with sodium phenobarbitone (PB) or 3-methylcholanthrene (3-MC). PB and 3-MC are cytochrome P450-specific inducers. The rates of formation of trichloroethylene and tetrachloroethane are presented in Table 4. Liver microsomes from PB-treated rats metabolized pentachloroethane to trichloroethylene (96%) and tetrachloroethane (4%). PB pretreatment resulted in a large induction of trichloroethylene and tetrachloroethane formation, whereas 3-MC pretreatment had no significant effect. Carbon monoxide, a high affinity cytochrome P450 inhibitor, effectively inhibited the metabolism (99% inhibition) of pentachloroethane, confirming that cytochrome P450 enzymes are the primary enzymes involved in pentachloroethane metabolism. Metyrapone (10^{-4} M) inhibited formation of trichloroethylene and tetrachloroethane by $47 \pm 10\%$, $27 \pm 6\%$ and $22 \pm 3\%$ for PB-treated rats, 3-MC-treated rats, and untreated controls, respectively. This study clearly indicates that the major metabolites of pentachloroethane are trichloroethylene and tetrachloroethane, and that the metabolism is catalyzed by PB-inducible cytochrome P450 isoforms.

TABLE 4. Rates of Formation of Pentachloroethane Metabolites by Rat Liver Microsomes Induced by PB or 3-MC

Pretreatment	Cytochrome P450 (nmol/mg protein)	Pentachloroethane (0.5 mM)	
		Trichloroethylene (nmol/mg protein/min)	Tetrachloroethane (nmol/mg protein/min)
PB	2.2	28.5 ± 1.7	1.20 ± 0.04
3-MC	1.1	4.2 ± 0.5	0.54 ± 0.08
Control	0.75	6.4 ± 0.5	0.64 ± 0.07

Results are mean \pm SD from 3 experiments

Yllner (1971) evaluated the metabolism and elimination of pentachloroethane in female NMRI mice. Pentachloroethane was administered subcutaneously at doses of 1.1-1.8 g/kg, and excretion was monitored for 3 days. Over the course of 3 days, approximately 32% of the administered dose was excreted unchanged via urine; 23.5% of the dose was excreted as trichloroethanol; 16.1% of the dose was excreted as trichloroacetic acid. In expired air, 7.4% of the dose was excreted as tetrachloroethane and 8.5% as trichloroethylene. Trichloroethanol and trichloroacetic acid are urinary metabolites produced during hepatic oxidation of trichloroethylene and tetrachloroethane.

Goldsworthy et al. (1988) evaluated the role of protein droplet accumulation in the renal carcinogenicity of male rats exposed to pentachloroethane. Male and female F344 rats were gavaged with 150 mg/kg pentachloroethane for 10 days. On day 10, the rats were euthanized and histopathology of kidney was carried out. Pentachloroethane caused significant increases in kidney protein droplet accumulation and immunohistochemical detection of α_{2u} -globulin protein in male rats, but not female rats. Proximal tubule sections were assessed for cell proliferation using a labeling index (percentage of labeled cells). The labeling index was not affected by pentachloroethane treatment in female rats. Male rats exhibited statistically significant increases in labeling index compared to male control rats. The authors hypothesized that increased incidences of renal tumors in male rats following pentachloroethane exposure are caused by nephrotoxicant-induced increases in cell replication. In summary, the nephrotoxic

effects of the pentachloroethane can be concluded to arise from an accumulation of alpha_{2u}-globulin, a mode of action that is not relevant to humans (U.S. EPA, 1991a).

NTP (1983) reported increases in protein hyaline droplet accumulation in the renal tubules of male rats exposed to pentachloroethane. The male rats exhibited nephrotoxic effects including severe tubular dilation in the pars recta (inner cortex), giant cells and casts in the dilated tubules, significantly increased incidence of mineralization of the renal papilla, and prominent interstitial fibrosis and interstitial accumulation of mononuclear inflammatory cells. These nephrotoxic effects have been associated with alpha_{2u}-globulin-characteristic nephropathy that is typically only observed in male rats. These renal effects are also frequently associated with a spontaneous age-related nephropathy syndrome (i.e., not related to alpha_{2u}-globulin) commonly referred to as chronic progressive nephropathy. However, NTP (1983) suggested that the nephrotoxic effects observed with exposure to pentachloroethane were distinct from chronic progressive nephropathy based on the severity of effects and the presence of giant cells within the tubules which are not characteristic of aging nephropathy. Goldsworthy et al. (1988) also reported significant increases in protein droplet accumulation. The alpha_{2u}-globulin protein was identified within the hyaline droplets via immunohistochemical staining. Evidence of cell proliferation in the proximal tubules as shown by significant increases in labeling index was observed in male rats administered pentachloroethane.

The EPA (U.S. EPA, 1991a) established criteria for determining if alpha_{2u}-globulin nephropathy is acting as a factor in the development of renal tumors. Positive evidence in three categories is required: increased number and size of hyaline droplets in renal proximal tubule cells; accumulating protein in the hyaline droplets is identified as alpha_{2u}-globulin; and additional aspects of the pathological sequence of lesions associated with alpha_{2u}-globulin nephropathy are present. The evidence in male rats suggests that the nephrotoxic effects of pentachloroethane may arise from an accumulation of alpha_{2u}-globulin and may not be relevant to humans. However, considering that the pathological sequence of lesions has not been fully established, the latter conclusion cannot be stated with certainty.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR PENTACHLOROETHANE

Chronic and subchronic (NTP, 1983) studies following oral exposure are available. ANOEL and LOAEL (adjusted for exposure regimen) for pentachloroethane were determined to be 89 and 180 mg/kg-day, respectively, for a 15% reduction in body-weight gain in both male and female rats in the 13-week NTP (1983) study. A similar reduction of 14% in female mice at 71 mg/kg-day is judged to be non-significant given the apparently random fluctuations in body weight gain for animals dosed at lower levels. The 29% body weight gain reduction for female mice at 357 mg/kg-day is considered to be biologically significant. In addition, no effect on body weights for either mice or rats were observed in the chronic study at doses up to 107 mg/kg-day (NTP, 1983). The subchronic mouse study suggests an equivocal NOAEL of 71 mg/kg-day, with a LOAEL of 357 mg/kg-day for reduction in body weight gain. The latter could also be considered to be a FEL, given the death of one animal at that dose. In addition, given the FEL of 180 mg/kg-day for mice in the 2-year NTP (1983) study, an overall NOAEL

and LOAEL for the pentachloroethane for mice cannot be determined. The FEL of 54 mg/kg-day for mortality in rats and mice in the chronic study (evident for rats as early as 18 weeks) confounds the derivation of subchronic and chronic p-RfDs, given the close proximity to the subchronic NOAELs (71 and 89 mg/kg-day for mice and rats, respectively). Thus, the data are considered to be inadequate for the quantitative derivation of subchronic and chronic p-RfDs.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR PENTACHLOROETHANE

Subchronic and chronic provisional RfCs cannot be derived for pentachloroethane because no toxicology information from the inhalation route of exposure is available. A route-to-route extrapolation could not be performed because of the lack of information on the absorption, metabolism and distribution of pentachloroethane following inhalation exposure.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR PENTACHLOROETHANE

The derivation of oral cancer slope factors was based on the female mouse hepatocellular adenomas observed in the chronic bioassay conducted by NTP (1983). Tumor incidences from this study are reported in Table 2. Data from male mice could not be used due to the high mortality in the high dose group, which precluded an evaluation of their lifetime incidences of hepatocellular carcinoma and adenoma. Hepatocellular carcinomas in female mice were statistically significantly increased in both dose groups compared to control. Hepatocellular adenomas in female mice exhibited a statistically significant dose-related increase in incidence. The combined data set will be used for derivation of oral cancer slope factors.

NTP (1983) concluded that: "Under the conditions of this bioassay, technical grade pentachloroethane containing 4.2% hexachloroethane (a known carcinogen in mice) was not carcinogenic in F344/N rats. The decreased survival of dosed rats might have reduced the sensitivity for a carcinogenic response in this species. Pentachloroethane was nephrotoxic to male rats. Technical grade pentachloroethane was carcinogenic for B6C3F1 mice, causing hepatocellular carcinomas in males and females, and adenomas in females."

Pentachloroethane was not mutagenic in Ames *Salmonella typhimurium* mutagenicity tests, both in the presence and absence of an exogenous metabolic activation system (NTP, 1983). It was also reported to be negative in CHO cells for chromosome aberrations (Galloway et al., 1987). Pentachloroethane has been observed to cause other forms of genotoxicity such as sister chromatid exchanges (Galloway et al., 1987), polyploidy (Matsuoka et al., 1996), and clastogenicity (Sofuni et al., 1996). Based on this evidence, it is unclear if pentachloroethane is a mutagenic carcinogen.

The tumorigenicity observed in male and female mice along with some evidence of genotoxicity suggests that pentachloroethane is "*Likely to Be Carcinogenic to Humans*" according to U.S. EPA Guidelines for Carcinogen Risk Assessment (2005a). Since the mode of

action for pentachloroethane carcinogenicity cannot be determined due to the limited information available, the default linear extrapolation approach was used to calculate the oral slope factor (U.S. EPA, 2005a). The application of age-dependent adjustment factors for early-life exposure when a mutagenic mode of carcinogenic action has been determined is not recommended. The mode of action of pentachloroethane is unknown and evidence for a mutagenic mode of action is not available.

The oral slope factor was derived using the combined incidence of hepatocellular adenomas and hepatocarcinomas in female B6C3F1 mice (NTP, 1983), assuming that the adenomas are direct precursors to the carcinomas. The doses used in this study (0, 250 mg/kg, and 500 mg/kg; 5 times/week) were first adjusted for continuous exposure by adjusting for exposure regimen (from experimental gavage dosing for 5 days per week to 7 days per week), resulting in continuous doses of 0, 178.5 and 357.1 mg/kg-day. These continuous doses were scaled to human doses by multiplying the continuous animal dose with the animal:human body weight ratio to the $\frac{1}{4}$ power: $(\text{animal weight}/\text{human weight})^{1/4}$, according to U.S. EPA (2005a). The human equivalent doses (HEDs) are 0, 25.7 and 51.4 mg/kg-day, respectively, assuming (default) body weights of 0.03 kg for mice and 70 kg for humans. The combined tumor incidences were adjusted for survival (early death of tumor-free animals) by the poly-3 method (Bailer and C.J. Portier, 1988). The poly-3 adjusted tumor incidences for these doses were 3/44, 36/40 and 32/34, respectively.

U.S. EPA Benchmark Dose Software version 1.4.1 was used for determination of the point of departure (U.S. EPA, 2007b). The dichotomous 1st order cancer multi-stage model was applied to the data (U.S. EPA, 2000). The model fit was adequate, with a Chi-square p-value of 0.117. The $\text{BMDL}_{\text{HED}/10}$ was 1.13 mg/kg-day, with a cancer slope factor of $0.0883 \text{ (mg/kg-day)}^{-1}$ (see Appendix A).

The oral cancer slope factor (p-OSF) for pentachloroethane is 0.09 per mg/kg-day.

The oral cancer slope factor for pentachloroethane should not be used with exposures exceeding the point of departure (1 mg/kg-day; $\text{BMDL}_{\text{HED}/10}$), because above this point the slope factor may not approximate the observed dose-response relationship adequately.

The presence of hexachloroethane in the dosing preparation is a confounding factor in this assessment. Hexachloroethane produces liver tumors in mice but not in rats. Therefore, at least some of the tumor response observed for the pentachloroethane-treated mice could be a result of hexachloroethane exposure. However, it is unlikely that hexachloroethane is a major factor in the observed tumorigenic response in this study. Hexachloroethane comprises only about 4% of the technical pentachloroethane material and the OSF for hexachloroethane is $0.014 \text{ (mg/kg-day)}^{-1}$ (U.S. EPA, 1987) which is much less than the OSF estimated for pentachloroethane. Therefore, the OSF for pure pentachloroethane is unlikely to be much less than the estimate of 0.09 per mg/kg-day.

Provisional inhalation unit risk estimates (p-IUR) could not be derived for pentachloroethane because of the lack of inhalation toxicology data, and the lack of information

on the absorption, metabolism, and distribution of pentachloroethane after inhalation exposure. A route-to-route extrapolation cannot be performed without this information.

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Appendix A: Benchmark Dose Derivations

Appendix A contains the output from the Benchmark Dose Software version 1.4.1 for the combined hepatocellular adenoma and hepatocarcinoma tumor incidence in female mice (NTP, 1983; U.S. EPA, 2007b).

Multi-stage 1° Polynomial

```
=====
Multistage Cancer Model. (Version: 1.5; Date: 02/20/2007)
Input Data File: C:\BMDS\PENTACHLOROETHANE.(d)
Gnuplot Plotting File: C:\BMDS\PENTACHLOROETHANE.plt
Wed Sep 26 21:07:41 2007
=====
```

BMDS MODEL RUN

The form of the probability function is:

$$P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1)]$$

The parameter betas are restricted to be positive

Dependent variable = r_comb
Independent variable = HED

Total number of observations = 3
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 = 0.296723
Beta(1) = 0.0538105

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.38
Beta(1)	-0.38	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0700696	*	*	*
Beta(1)	0.0706968	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-31.5618	3			
Fitted model	-32.6066	2	2.08954	1	0.1483
Reduced model	-79.3336	1	95.5436	2	<.0001

AIC: 69.2131

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled		
			Observed	Size	Residual
0.0000	0.0701	3.083	3	44	-0.049
25.7600	0.8495	33.980	36	40	0.893
51.3700	0.9754	33.163	32	34	-1.287

Chi^2 = 2.46 d.f. = 1 P-value = 0.1170

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1.49031

BMDL = 1.13237

BMDU = 1.97227

Taken together, (1.13237, 1.97227) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0883104

