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TOXICOLOGICAL REVIEW

OF

HEXACHLOROCYCLOPENTADIENE

(CAS No. 77-47-4)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response assessment in IRIS pertaining to chronic exposure to hexachlorocyclopentadiene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of hexachlorocyclopentadiene.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrapulmonary or systemic effects). It is generally expressed in units of mg/m³.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m³ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identifications and dose-response assessments for hexachlorocyclopentadiene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996), *Interim Policy for Particle Size and Limit Concentration Issues and Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995a), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998a), and a memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

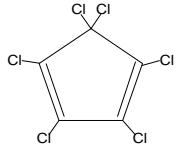
Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Other names for hexachlorocyclopentadiene include C-56, hexachloro-1,3-cyclopentadiene, graphlox, HCCP, HCCPD, Hex, hexachloropentadiene, HRS 1655, NCI-C55607, PCL, and perchlorocyclopentadiene. It is predominantly used as an intermediate in the production of many dyes, resins, pharmaceuticals, flame retardants, insecticides, and polyester resins. Hexachlorocyclopentadiene (HCCPD) is also used to produce ketones, fluorocarbons, acids, esters, and shockproof plastics.

HCCPD exists as a dense oily liquid, pale yellow to amber in color, at room temperature (melting point at -9°C , boiling point at 239°C). It has a pungent, unpleasant odor. Vapors are present at room temperature because of its high vapor pressure. HCCPD is soluble in organic nonpolar solvents but only slightly soluble in water. HCCPD degrades in the presence of light and may decompose to produce toxic fumes upon heating (HSDB, 1999). See Table 1 for selected chemical and physical properties of HCCPD.

Table 1. Chemical and physical properties of hexachlorocyclopentadiene

Properties	Values	Reference
Boiling point	239°C	HSDB, 1999
Melting point	-9°C	HSDB, 1999
Molecular weight	272.77	HSDB, 1999
Density	1.7019 at 25°C	HSDB, 1999
K _{oc}	4,265	U.S. EPA, 1999
Log K _{ow}	3.99	U.S. EPA, 1999
Solubility	2 mg/L water at 25°C	U.S. EPA, 1995b
Vapor pressure	0.08 mm Hg at 25°C	U.S. EPA, 1999
Henry's law coefficient	2.7×10^{-2} atm-cu m/mole	U.S. EPA, 1999
Chemical structure (C ₅ Cl ₆)		

Conversion factor: 1 ppm = 11.3 mg/m³; 1 mg/m³ = 0.088 ppm (World Health Organization, 1991).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

Yu and Atallah (1981) showed that HCCPD is poorly absorbed in rats following gavage administration. They administered single doses of 99% pure ^{14}C -HCCPD to 21 female and 6 male Sprague-Dawley rats (divided into groups of 1–3 per dose and time-point of analysis). Approximately 25 mg/kg ^{14}C -HCCPD dissolved in 0.5 mL corn oil was delivered via gavage while 0.73 mg/kg HCCPD dissolved in 0.3 mL 20% Emulphor EL0620 saline solution was administered IV. Urine and fecal samples were obtained daily. Blood samples were obtained at various postadministration durations, with the first sample taken at 0.5 hours and the last sample taken at 24 hours.

The concentration of ^{14}C in blood following oral administration rose gradually to a maximum of 2.25 ± 0.30 ppm HCCPD-equivalents at 4 hours postdosing, and then fell to 0.95 ± 0.16 ppm by 24 hours postdosing. After IV administration, HCCPD equivalents reached a maximum of 5.08 ± 1.02 ppm at 0.5 hours postexposure and dropped to 2.34 ± 0.75 ppm at 24 hours. Despite the much lower dose given by IV, the area under the concentration-duration curve for the blood of IV-injected rats was 70 times that of gavaged rats. The body burden for IV-injected rats was 10 times that of gavaged rats.

Lawrence and Dorough (1981) used one female Sprague-Dawley rat/dose to investigate the retention of 1.4, 17.3, and 37.4 μg ^{14}C -HCCPD /kg inhaled, via nose-only exposure, over a 1-hour period. Retention of the compound was 84% and independent of dose. Lawrence and Dorough (1982) performed a similar experiment and reported 91% retention after 1.5 hours and 95% retention after 2 hours inhalation exposure to both low (1-5 $\mu\text{g}/\text{kg}$) and high (30-40 $\mu\text{g}/\text{kg}$) doses of ^{14}C -HCCPD. Lawrence and Dorough (1982) also measured the blood concentrations of ^{14}C after administration of 10 μg ^{14}C -HCCPD/kg via 0.5 mL corn oil gavage, nose only inhalation (1 hour), and IV routes (in 0.2 mL dimethyl sulfoxide or 10:4:1 saline:propylene glycol:ethanol) and confirmed the results of Yu and Atallah (1981) which indicated poor absorption for the oral route. Peak ^{14}C blood concentrations for the oral route were approximately 1/5th those of the inhalation route and approximately 1/50th those of the IV route.

3.2. DISTRIBUTION AND METABOLISM

Several studies were performed to determine distribution and metabolism of HCCPD after oral administration. Mehendale (1977) administered 5 μmole (~ 6 mg/kg in rats weighing 225–250 g) of ^{14}C -HCCPD in 0.2 mL corn oil to male Sprague-Dawley rats via oral intubation. Urine and fecal samples were collected daily for 7 days. The animals were then sacrificed for collection of liver, kidneys, fat, lung, muscle, and blood tissues. After 7 days, the kidneys retained 0.5% of the administered dose, the liver retained less than 0.5%, and the remaining tissues contained only trace amounts. Thin-layer chromatography of organic urine extracts revealed four metabolites of HCCPD, which were not chemically characterized.

Yu and Atallah (1981) also investigated the distribution of HCCPD in rats dosed with 25 mg/kg ^{14}C -HCCPD by gavage or 0.73 mg/kg by IV injection. Brain, heart, lung, muscle, fat, gonad, uterus, spleen, kidney, liver, blood, digestive system, skin, hair, and urinary bladder were analyzed for retained radiolabel at 8, 24, 48, or 72 hours after oral administration. In gavaged rats, the kidney contained 16.20 ppm HCCPD equivalents, whereas the liver retained 6.23 ppm, and the gonad, fat, lung, and blood retained between 1.28 and 1.89 ppm equivalents at 8 hours postdosing. All other tissues had less than 1 ppm. At 24 and 48 hours postdosing, the kidney and liver still had the highest concentrations of HCCPD equivalents.

Tissue concentrations were measured at 24 and 48 hours after IV administration (Yu and Atallah, 1981). Again, the kidney retained the highest concentration (2.64 ppm) of ^{14}C -HCCPD equivalents at 24 hours after administration. The blood, spleen, and liver, in this order, contained the next highest concentrations. At 48 hours after IV administration, spleen and blood concentrations were the highest (about 2.95 ppm), followed by the kidney at 2.02 ppm. All other tissues contained less than 0.42 ppm.

These data indicate that the tissue distribution of HCCPD and its metabolites was similar from 8 to 72 hours after oral administration, with HCCPD primarily retained in the kidney and liver. After IV administration, HCCPD and its metabolites were distributed primarily in the kidney, but the blood, spleen, and liver also had relatively high concentrations. The study shows that although the distribution of HCCPD and its metabolites varies somewhat with route of administration, the kidney and liver are the major organs of concentration for both oral and IV routes. When the tissue concentrations are considered in proportion to the dose received, the data also indicate that HCCPD and its metabolites are retained longer after IV administration than after oral administration. The authors suggest that lower retention of orally dosed HCCPD is due to its poor absorption in the gut.

Based on blood data from IV dosed rats, Yu and Atallah (1981) developed an open two-compartment pharmacokinetic model. The model proposed that HCCPD was rapidly metabolized and distributed in the central compartment (blood, liver, kidney, and lung) and then gradually redistributed to the peripheral compartment (fat tissues) after IV injection. Comparison of observed to expected values for radiolabel concentration in blood showed a good agreement. Using the model, the authors predicted a biological half-life of 32 hours for HCCPD in the rat (Yu and Atallah, 1981). No modeling was performed for oral administration.

Lawrence and Dorough (1981) investigated differences in distribution between corn oil gavage and inhalation administration in female Sprague-Dawley rats. For inhalation studies, rats inhaled, via nose-only exposure, 24 μg ^{14}C -HCCPD/kg (exposure concentration not reported) for single 1-hour periods. For measurable tissue levels of ^{14}C , the gavage dose had to be much higher, 6 mg/kg ^{14}C -HCCPD in 0.5 mL corn oil. Tissue samples were taken at 72 hours postdosing, combusted, and then $^{14}\text{CO}_2$ was trapped and counted. Radioactivity was measured in the trachea, lungs, liver, kidneys, and carcass. Levels were reported as a percentage of the administered radioactivity. After inhalation exposure, the carcass retained $7.8\% \pm 2.0\%$ of the dose, the lungs retained $2.0\% \pm 0.4\%$, the kidneys retained $0.8\% \pm 0.2\%$, and the liver retained

0.4% ± 0.2%. After gavage, the carcass retained 1.87% ± 1.16% of the dose, the kidneys retained 0.47% ± 0.06%, the liver retained 0.39% ± 0.06%, and other tissues retained less than 0.1% of the radiolabel. For either route, only trace amounts of radiolabel were found in fat.

In a similar study (Lawrence and Dorough, 1982), distribution of ¹⁴C-HCCPD in female Sprague-Dawley rats was studied following oral, inhalation, and IV administration. Doses were 6 mg/kg via gavage, 24 µg/kg for the inhalation route (via nose cone), and 10 µg/kg for the IV route. Trachea, lungs, liver, kidneys, fat, and remaining carcass were assayed for ¹⁴CO₂ at 72 hours postexposure. After inhalation exposure, the highest concentration of HCCPD equivalents was in the trachea (107 ± 65.0 ppb), followed by lungs (71.5 ± 55.2 ppb) and kidneys (29.5 ± 20.2 ppb). After oral exposure, the highest concentrations were in the kidneys (3,272 ± 84 ppb), liver (539 ± 72 ppb), and lungs (420 ± 250 ppb). Following IV exposure, the kidneys retained the highest concentration, 22.3 ± 0.6 ppm, while the lungs retained 14.9 ± 1.1 ppm and the liver retained 9.6 ± 1.1 ppm HCCPD equivalents. The trachea retained only 3.3 ± 1.7 ppm following IV administration. These data are consistent with those from Lawrence and Dorough (1981), showing that distribution depends upon route of administration, with oral and IV HCCPD resulting in generally similar distribution patterns. Oral and IV administration resulted in the highest concentrations of HCCPD equivalents in the kidneys and then in the liver and lungs, whereas inhalation exposure resulted in the highest concentrations in the trachea, followed by lungs and then kidneys. The concentration of HCCPD equivalents in fat was only appreciable for the oral route.

Results from a study of distribution of radiolabeled HCCPD by Dorough and Ranieri (1984) in rats and mice were consistent with those of Lawrence and Dorough (1982). Male and female Sprague-Dawley rats and mice were gavaged with 2.5 mg/kg or 25 mg/kg ¹⁴C-HCCPD (in 0.9 mL corn oil for rats and 0.2-0.3 mL corn oil for mice). After both doses, the kidney contained the highest concentration of radiolabel in the rat, but the liver contained the highest concentration in the mouse at 1 and 7 days after exposure. A study of the distribution of dietary HCCPD was performed using concentrations of 1, 5, and 25 ppm in food (Dorough and Ranieri, 1984). After 15 days on the diet, radioassay of tissues collected from female rats showed the highest concentration of HCCPD equivalents/dietary ppm in the kidneys, fat, then in the gonads and liver at all dietary dose levels. Male rats retained the compound in the same distribution pattern as the female rats, but had higher concentrations of HCCPD equivalents/ppm diet in the liver than in the gonads. Female and male mice retained the compound primarily in the fat, then the liver, then the gonads and kidney. Gonads concentrated radioactive residues at a comparable, but slightly lower, level to fat in both species, whereas muscle and brain did not accumulate appreciable amounts, even at the 25 mg/kg dose.

Yu and Atallah (1981) also studied the nature of the metabolites in the tissues by extracting tissue homogenates with organic solvents. The majority of degradation products were polar and were organically extractable only after acidification. Attempts to identify the metabolites of HCCPD in rodents (Yu and Atallah, 1981; Mehendale, 1977; Logan and Croucher, 1984) have been unsuccessful.

Yu and Atallah (1981) incubated fecal material from rats with aliquots of 315 μg of ^{14}C -labeled HCCPD to study the stability of HCCPD in this environment. Samples of the mixture were collected at 0, 1, 6, and 24 hours, homogenized, and organically extracted. The remaining solid was dried and radioassayed, while the organic extracts were partitioned using an acetonitrile/water mixture, and the layers were radioassayed. The results indicated that the HCCPD was rapidly degraded in the feces, with a half-life of 1.6 hours. The fact that antimicrobial compounds slowed the degradation indicated that microbial action was responsible for HCCPD breakdown in the fecal homogenate.

Samples taken from the contents of the duodenum and small and large intestine from selected rats were homogenized and added to radiolabeled HCCPD in the presence or absence of antimicrobial agents (Yu and Attalah, 1981). Sampling and extraction proceeded as described for the fecal homogenates. The results of intestinal incubation indicated that HCCPD degradation proceeded slowly in the gut in a microbe-dependent fashion with a half-life of 10.1 hours. Degradation rates of HCCPD by liver homogenates were similar for active ($t_{1/2} = 14.2$ hours) and denatured ($t_{1/2} = 12.4$ hours) homogenates. Because of the similarity of degradation rates between active and denatured extracts, the authors proposed that the necessary cofactors for proper liver enzyme activity to degrade HCCPD were likely not present in the prepared extracts, or that most of the degradation of HCCPD takes place outside of the liver.

El Dareer et al. (1983) performed *in vitro* binding experiments with HCCPD and varying biological materials obtained from rats to study the interaction of the compound with biological macromolecules. After an incubation of ^{14}C -HCCPD with the material for 0, 5, or 60 minutes, a series of organic extractions was performed. Liver homogenates, plasma, and whole blood incubated with the HCCPD formed virtually inextractable mixtures even at 0 minutes. Feces and intestinal contents, however, were easily extractable at 0 and 5 minutes, and extractability did not decrease until the 60-minute incubation. The results show the high chemical reactivity of HCCPD toward biological materials.

3.3. EXCRETION

In the study by Mehendale (1977), which gavaged rats with ~ 6 mg/kg ^{14}C -HCCPD, urine and fecal samples were collected daily for 7 days. After 7 days, approximately 33% of the total radioactivity was excreted in the urine, with 87% of that eliminated within the first 24 hours. Fecal excretion accounted for 10% of the administered dose, with 60% of fecal excretion occurring during the first day. Only trace amounts of radioactivity were recovered in feces after the third day. Because individual tissues contained less than 0.5% of the radioactivity and only 43% had been excreted in feces and urine, Mehendale (1977) suggested that HCCPD may be eliminated, to a large extent, in exhaled air.

In another experiment, Mehendale (1977) injected ~ 6 mg/kg ^{14}C -HCCPD into the femoral veins of male rats and collected samples of blood and bile at 15, 30, 45, and 60 minutes. The radioactivity in blood decayed biexponentially with a terminal half-life of 1 hour. Approximately 9% of the radioactivity was excreted in bile over 1 hour. Predosing the rats with

50 mg/kg/day HCCPD for 3 days by gavage had no effect on biliary excretion or on the decline of radioactivity in blood.

In the study by Yu and Atallah (1981) described in Section 3.1, urine and fecal samples were analyzed for radioactivity at 8, 24, or 48 hours after a single oral or IV dose of radiolabeled HCCPD. After gavage dosing, radiolabel was eliminated mainly in feces (70%) and urine (17%) within 48 hours. Fecal excretion after oral administration was much greater than that observed by Mehendale (1977). When administered intravenously, the radioactivity was eliminated equally in feces (21%) and urine (18%) over the same time period.

Lawrence and Dorough (1981) administered 5 μg ^{14}C -HCCPD/kg to female rats via 1-hour inhalation or by gavage to compare excretion by the two exposure routes. Urine and fecal samples were taken at 24, 48, and 72 hours postdosing. Radioactivity in urine samples was counted in a scintillation counter, while fecal samples were combusted and trapped $^{14}\text{CO}_2$ was assayed. At 24 hours after gavage, elimination was primarily in the feces ($62.2\% \pm 8.0\%$) as compared to the urine ($22.8\% \pm 1.8\%$). Fecal and urinary excretion after oral administration was similar to that observed by Yu and Atallah (1981). After inhalation exposure, elimination was higher in urine ($29.7\% \pm 4.5\%$) than in feces ($17.0\% \pm 7.5\%$). The proportions of urine:fecal excretion did not change at 48 or 72 hours. Another inhalation experiment (Lawrence and Dorough, 1981) in which rats were administered 1.4-37.4 μg ^{14}C -HCCPD/kg showed that excretion by exhalation was insignificant. Less than 1% of the radiolabel was eliminated as ^{14}C -HCCPD in expired air in the 24 hours following exposure, and no $^{14}\text{CO}_2$ was detected in expired air.

A follow-up study by Lawrence and Dorough (1982) compared the fate of inhaled (24 $\mu\text{g}/\text{kg}$), oral (5 $\mu\text{g}/\text{kg}$), and IV (10 $\mu\text{g}/\text{kg}$) ^{14}C -HCCPD in female Sprague-Dawley rats. Radiolabeled residues were primarily excreted via the feces after oral and IV routes, and primarily via the urine following inhalation exposure. After 3 days, the percentage of the dose eliminated via the feces was significantly higher for oral administration (~70%) than it was for IV (~30%) or inhalation (~27%). These results for percentage urinary excretion confirm those of Yu and Atallah (1981) and Lawrence and Dorough (1981). Lawrence and Dorough (1982) found total body burden was much higher after IV dosing ($31.0\% \pm 7.8\%$), as compared to oral ($2.8\% \pm 1.1\%$) or inhalation ($12.9\% \pm 4.7\%$) exposure. Biliary excretion of label was found to be highest following oral exposures, accounting for 18% of the dose in 28 hours. Biliary excretion of ^{14}C -HCCPD was 13% of the IV dose and ~9% of the inhaled dose.

In the Dorough and Ranieri (1984) study, female rats and mice intubated with a single low (2.5 mg/kg) dose of radiolabeled HCCPD excreted the majority of the label in feces as compared to urine at both 1 and 7 days postdosing. After 1 day, rats excreted 65.2% of the dose in feces and 12.4% in urine while mice excreted 42.1% of the dose in feces and 13.8% in urine. The percentage excretion was higher at 7 days with a similar feces:urine ratio. At 25 mg/kg, there were no appreciable differences between rats and mice in the amount of radioactivity excreted in feces vs. urine. Results from male rats treated with 25 mg/kg ^{14}C -HCCPD showed

that excretion was similar to that in females. Fecal excretion after 3 days was 73.6% of the administered dose while urinary excretion was 13.4%.

El Dareer et al. (1983) also investigated the disposition of ^{14}C -HCCPD administered to rats via a single oral gavage dose (4.1 mg/kg or 61 mg/kg ^{14}C -HCCPD) in 1 mL corn oil/150 g body weight, a single IV dose (0.59 mg/kg ^{14}C -HCCPD) in 0.15 mL 1:1:4 Emulphor EL-620:ethanol:water/150 g body weight, or a single inhaled dose (1.1 mg administered over 2 hours via whole-body exposure). Following oral doses, >90% of the radioactivity was excreted after 72 hours, with twice as much contained in the feces as in urine. Only 34% of the IV dose was excreted in the feces after 72 hours, with urinary excretion accounting for 15.8%, and 39.0% remaining in the tissues. At 6 hours following the inhalation exposure, excretion was primarily via the urine (41.0% of dose). The amount excreted via the feces (28.7%) was comparable to that remaining in tissues (28.9%). At 72 hours after inhalation, excretion was roughly equal between feces and urine (40%–50%), with only a small portion remaining in tissues (11%). El Dareer et al. (1983) essentially confirm the results for urinary and fecal excretion obtained by Lawrence and Dorough (1982) for oral, IV, and inhalation routes of exposure.

Another study investigated the excretion of HCCPD in rats, rabbits, and mice after the administration of 20 mg/kg radiolabeled HCCPD (Logan and Croucher, 1984). Rats and mice were dosed via gavage (2 mL corn oil/kg body weight), while rabbits were dosed via gelatin capsule. Consistent with the results of previous investigators (Yu and Atallah, 1981; Lawrence and Dorough, 1982; Dorough and Ranieri, 1984), fecal excretion of radiolabel was predominant, with urinary excretion secondary. By the end of 3 days, 85%–92% of the entire dose was eliminated. Urinary excretion was 20%, 23%, and 35% of the administered radiolabel for rats, mice and rabbits, respectively. Fecal excretion over the same period was 68%, 69%, and 51% for rats, mice, and rabbits, respectively. As also shown by Lawrence and Dorough (1981), little or no $^{14}\text{CO}_2$ was detected in expired air (measured for rats only). After IV administration of 24 mg/kg radiolabeled HCCPD (200 mg/mL in 30 μL ethanol) to a separate group of rats, an equal percentage of the dose administered was excreted in the feces (10%) and urine (9%), with much less of the total dose excreted (19%) at the end of 3 days. The equal proportion of fecal vs. urinary excretion was similar to other studies using IV administration, but the percentage of the total dose excreted was much less than that found in other studies (Yu and Atallah, 1981; Lawrence and Dorough, 1982; El Dareer, 1983).

Most of these metabolism studies indicate that excretion of HCCPD metabolites varies depending on exposure route. Fecal excretion predominates after oral exposure, but urinary excretion predominates following inhalation exposure. Microbial metabolism to polar metabolites in the gut is likely to be responsible for the large proportion of fecal excretion after oral administration. Fecal and urinary excretion are approximately equal after IV administration. HCCPD metabolites produced following inhalation exposure are retained in the bodies of rodents longer than those from ingested HCCPD, which may indicate that the metabolism to polar compounds occurs more slowly after inhalation exposure.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. **Buncher, CR; Moomaw, C; Sirkoski, E. (1980) Mortality study of Montague plant. Unpublished report for Hooker Chemical Corporation. Doc. # 878212111. NTIS/OTS84003A.**

Buncher et al. (1980) conducted an occupational mortality study with 341 workers at the Hooker Chemical Corporation plant in Montague, MI. The plant produced HCCPD and other chlorinated hydrocarbons. Three hundred forty-one employees who had worked at least 90 days between October 1, 1953, and December 31, 1974, were included in the cohort. Follow-up was through December 31, 1978. Expected deaths were determined using sex-, age- and year-specific U.S. mortality rates. The 24 deaths, grouped in such causal categories as all causes, all cancers, diseases of the circulatory system, diseases of the digestive system, and external causes, were fewer than expected. The six observed cancer deaths included one cancer each in the esophagus, large intestine, breast, and kidney, and two of the respiratory system. The authors indicate that the ratios of observed to expected deaths for the respiratory cancers (0.87) and colon cancer (1.75) are not statistically unusual. The remaining cancers have ratios greater than or equal to 5; however, the small numbers of deaths prevent drawing a firm conclusion. The short follow-up period in this study is also a limitation.

4.1.2. **Wang, HH; MacMahon, B. (1979) Mortality of workers employed in the manufacture of chlordane and heptachlor. J Occup Med 21:745-748.**

This retrospective mortality study involved white male workers from the Velsicol Chemical plants in Marshall, IL, and Memphis, TN. The population studied consisted of 1,403 white males currently or formerly employed for more than 3 months during the years 1946–1975 for the Illinois plant and 1952–1976 for the Tennessee plant. The plants manufactured heptachlor and chlordane, for which HCCPD is an intermediate, during those periods. Approximately 34% of the subjects had less than 10 years follow-up and 36% had 20 or more years follow-up. Expected deaths for these person-years were calculated from white male national mortality rates through 1975. Observed deaths due to all causes were significantly lower than expected deaths. Deaths due to cerebrovascular disease, however, were significantly elevated over those expected. Because exposure to several organochlorines occurred, the increase in cerebrovascular disease could not be attributed to HCCPD exposure. Deaths due to all cancers were less than expected, but deaths due to lung cancer were greater than expected, although not significantly. Lung cancer deaths were not associated with duration of employment or duration of follow-up, but the numbers available for such analysis were small. No data on individual cigarette smoking habits were available. There was one death each from cancer of the liver, bladder, prostate, and central nervous system.

4.1.3. Shindell and Associates. (1980) Report of epidemiologic study of the employees of Velsicol Chemical Corporation plant, Marshall, Illinois, January 1946–December 1979. Unpublished report for Velsicol Chemical Corporation, July 1980. Doc. # 40-8149074.

Shindell and Associates (1980) conducted a mortality study of 783 workers employed at least 3 months between January 1, 1946, and December 31, 1979, at the Velsicol Chemical Corporation plant in Marshall, IL. The aim of the study was to evaluate the overall health status of all former and current employees with 3 months or longer employment during a time when the Marshall plant was manufacturing chlordane. This cohort is similar to that studied by Wang and MacMahon (1979), but included nonwhite males and women. The cohort included 783 individuals comprising 689 white males, 10 nonwhite males, and 84 females. The two studies employed different follow-up techniques. The vital status of 97.4% of the cohort was known. The causes of death examined included all deaths, malignant neoplasms, diseases of the heart and circulatory system, cerebrovascular disease, trauma, and others. The number of observed deaths in each category was compared to the number of expected deaths calculated from race- and sex-specific U.S. mortality rates for appropriate 5-year periods. No excess deaths related to any specific job class or product were seen. Except for "other deaths" in females, the number of deaths observed was lower than the number expected. The 22 deaths from cancer included brain, kidney, liver, lung, and digestive system cancers. Eight of the 22 cancer deaths were from lung cancer. The number of expected deaths for each of these specific cancers was not calculated. This study reported no significant differences between mortality of plant employees and individuals from the U.S. population matched for race, age, and sex during the time period the cohort was studied. The authors noted the healthy worker effect in mortality data from the Marshall plant.

4.1.4. Shindell and Associates. (1981) Report of epidemiologic study of the employees of Velsicol Chemical Corporation plant, Memphis, Tennessee, January 1952–December 1979. Unpublished report for Velsicol Chemical Corporation, March 1981. Doc. # 40-8149074.

The second mortality study performed by Shindell and Associates involved the Velsicol plant in Memphis, TN. The cohort included 1,115 employees with a minimum of 3 months of employment between January 1952 and December 31, 1979. The purpose of the study was to evaluate the overall health status of all former and current employees with 3 months or more employment during a time when the plant was manufacturing heptachlor. The study design was the same as Shindell and Associates (1980). The vital status of 92.8% of the cohort was known. Consistent with the earlier Shindell study, this investigation revealed no significant differences between mortality of plant employees and the overall U.S. population. Deaths from strokes and from trauma showed an insignificant increase over the number of expected deaths. The distribution of the standard mortality ratio of deaths by site of cancer and job class showed a nonsignificant excess of lung cancer in maintenance workers. The authors concluded that there was no pattern of neoplasia suggestive of job-related risk. In addition, mortality by cause was consistent regardless of tenure of employment at other plants.

4.1.5. Brown, DP; Ditraglia, D; Namekata, T; et al. 1980. Mortality study of workers employed at organochlorine pesticide manufacturing plants. U.S. Dept of Health, Education and Welfare and University of Illinois. Unpublished report. May 1980. Doc. # 40-8149074

This mortality study involved cohorts from four different chemical plants that manufactured organochlorine pesticides. The cohorts were defined as all workers at each plant who had worked at least 6 months prior to December 31, 1964. Causes of deaths that occurred prior to December 31, 1976, were recorded. The entire study included about 2,100 individuals, but the cohorts at each plant were evaluated separately. These cohorts overlapped the one used in the Wang and MacMahon study (1979) but extended the follow-up period. Observed deaths in the cohorts were far fewer than expected, reflecting the healthy worker effect. The expected value was calculated using U.S. white-male cause-specific mortality rates, but the report did not specify the sex or ethnicity of the employees. The increase in cerebrovascular disease observed in the Wang and MacMahon study (1979) was not reported in this study. A decrease in expected deaths from all malignant neoplasms in each plant was observed, but it was not statistically significant. There were slight, but not statistically significant, increases in stomach cancer deaths in one plant, and slight excesses of cancers of the esophagus, rectum, liver, and lymphatic and hematopoietic systems in another plant. Exposure to multiple organochlorine compounds in each of the plants precludes linking these cancer cases with exposure to HCCPD or any other individual compound.

4.1.6. Kominsky, JR; Wisseman, CL, III; Morse, DL. (1980) Hexachlorocyclopentadiene contamination of a municipal wastewater treatment plant. Am Ind Hyg Assoc J 41:552-556.

This report documents an accidental acute occupational exposure to high concentrations of HCCPD when an unidentified odoriferous and viscous substance accumulated on the bar screens and grit collection systems of a wastewater treatment plant. When employees used steam to remove the substance, a blue haze was generated and permeated the primary water treatment area, forcing approximately 20 workers to seek medical attention for tracheobronchial irritation. On the following day, after a heavy rain, personnel noticed a similar blue haze over the grit collection channels accompanied by an offensive odor throughout the primary treatment area. The plant was closed 2 days later when HCCPD and octachlorocyclopentene (OCCP) were detected in the wastewater. Airborne concentrations of HCCPD and OCCP during the exposure period were not known, but 4 days after the plant was closed for cleaning, concentrations in the screen and grit chambers were 270-970 ppb, and HCCPD concentrations in the blue haze were as high as 19,200 ppb (217 mg/m³)¹.

Of the 177 treatment plant employees (23 females, 154 males) who responded to a medical questionnaire, 59% reported symptoms of eye irritation, 45% reported headaches, and

¹Calculated using conversion of 1,000 ppb = 11.3 mg/m³.

27% reported throat irritation. Six weeks after exposure to the organochlorines, many complaints of persistent health effects were reported: headache (18%), persistent fatigue (15%), chest discomfort (13%), skin irritation (10%), and cough (9%). A review of the medical records of 90 employees who were observed by the plant physician over a 2-month period starting with the first reports of contamination revealed symptoms of headache as well as mucous membrane and respiratory tract irritation. Unusual symptoms were reported by individuals with acute, high-level exposure to the compounds, including one report of “burning feet” (the individual’s boots deteriorated in contaminated sludge), three incidences of “sunburn-like” facial irritation, seven reports of rashes on exposed skin, and seven reports of transient confusion or memory loss. No changes were observed for the 28 employees who received chest X-rays. Arterial blood gas analyses were performed for 16 of the 28 employees and pulmonary function tests were performed for 22 people. Neither test revealed abnormalities.

Laboratory tests from 97 cleanup crew members revealed no significant abnormalities in renal function, complete blood counts, or urinalyses; however, 18 cleanup workers had mild liver function abnormalities exhibited by abnormal serum values in glutamate-oxaloacetate transaminase, alkaline phosphatase, total bilirubin, and/or lactate dehydrogenase. The proportion of the 18 workers that underwent preexposure monitoring is uncertain because the authors indicate only that 52 of the 97 cleanup workers were monitored prior to exposure. Thus, the relationship of the abnormal liver indices to exposure is uncertain. However, seven persons did have increased serum glutamate-oxaloacetate transaminase that seemed to be temporally related to exposure to contaminated sewage. The authors concluded that exposures to HCCPD and associated compounds may produce liver damage. The association of HCCPD exposure and liver function abnormalities is confounded, however, by the lack of information on preexposure monitoring and coexposure to OCCP.

4.1.7. Boogaard, PJ; Rocchi, PSJ; van Sittert, NJ. (1993) Effects of exposure to low concentrations of chlorinated hydrocarbons on the kidney and liver of industrial workers. Br J Ind Med 50:331-339.

In this study, 73 male operators in a chemical plant that produced several different chlorinated hydrocarbons were evaluated for liver and kidney toxicity. The subjects were employed for an average of 8.2 years (0.5–23 years). A control group consisted of 35 male employees who were not occupationally exposed to the chemicals. The control group was well matched to the exposed population in all selected parameters except age. Age was a confounding factor for several of the biochemical analyses performed.

Exposure to HCCPD, allyl chloride, 1,3-dichloropropene, and epichlorohydrin was measured by personal samplers on a few individuals. While mean concentrations of 1,3-dichloropropene and epichlorohydrin were well below the applicable occupational exposure standards (5 and 4 mg/m³, respectively), exposures to allyl chloride and HCCPD occasionally exceeded the maximum allowable concentrations of 3 and 0.11 mg/m³, respectively. Individual exposures could not be estimated because personal samplers were used on few employees.

Biochemical analyses indicated no differences between the control and exposed populations on any of the liver function tests (serum alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, gamma-glutamyltranspeptidase, lactate dehydrogenase, and total serum bile acids). Further, no statistically significant differences were observed in kidney function tests measuring urinary levels of alanine aminopeptidase, N-acetyl- β -D-glucosaminidase, retinol binding protein, and total protein. The exposed group had significantly greater urinary albumin levels than did controls (8.09 mg/g vs. 4.68 mg/g), but the levels were within normal limits. Boogaard and Caubo (1994) showed that shift workers have increased albumin excretion compared with employees who work only during the day, and suggested that this may be a circadian effect. Thus, the results of Boogaard et al. (1993) indicate that exposure to occupational concentrations of these chlorinated hydrocarbons does not cause significant liver or kidney damage.

4.2. SUBCHRONIC/CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS— INHALATION AND ORAL

4.2.1. Inhalation Studies

4.2.1.1. *NTP. (1994) Toxicology and carcinogenesis studies of hexachlorocyclopentadiene in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program Technical Report Series 437:318.*

In 13-week range-finding studies in F344/N rats and B6C3F1 mice, NTP exposed groups of animals (10 per sex per species) for 5 days per week, 6 hours per day, to atmospheres containing 0, 0.04, 0.15, 0.4, 1, or 2 ppm HCCPD (0, 0.45, 1.7, 4.5, 11, or 22 mg/m³, respectively). Standard bioassay data including body weights, clinical chemistry, hematology, urine analysis, organ weights, pathology, and histopathology were collected. All rats in the 11 and 22 mg/m³ groups died within 4 weeks. Clinical effects in rats included listlessness in the 22 mg/m³ group from week 1, in the 11 mg/m³ group from week 2, and in the 4.5 mg/m³ group during week 3. Rats in the 11 and 22 mg/m³ groups also experienced respiratory distress (mouth breathing and increased respiration rate). Male rats in the 4.5 mg/m³ dose group exhibited a statistically significant decrease in body weight compared with controls, but the decrease is not considered to be toxicologically significant because it was less than 10%. Body weights of treated female rats were similar to controls. No other treatment-related clinical findings of toxicity were reported.

Necropsy of rats in the 11 and 22 mg/m³ groups revealed extensive coagulation necrosis in the respiratory epithelium of the nose, larynx, trachea, bronchi, and bronchioles. Necrosis was accompanied by inflammatory signs such as vascular congestion, edema, fibrin accumulation, and neutrophil and mononuclear cell infiltration. Male rats in the 4.5 mg/m³ group exhibited necrotizing and suppurative inflammation of the nose, bronchus, and bronchioles and squamous metaplasia of the nose, as well as increased lung weights. The squamous metaplasia was focal in nature, generally observed on the tips of the turbinates, and characterized by stratification of the epithelium to form three to four poorly defined layers of flattened, nonkeratinized polygonal

cells. Female rats seemed to be less sensitive. At the 4.5 mg/m³ exposure, the only nose effect was suppurative inflammation, and fewer females than males exhibited necrotizing and suppurative inflammation of the bronchus and bronchioles. Because no respiratory lesions were seen at exposures lower than 4.5 mg/m³ HCCPD, the NOAEL for rats was 1.7 mg/m³ and the LOAEL was 4.5 mg/m³.

All mice in the 11 and 22 mg/m³ groups died within 5 weeks. Before the end of the study, seven deaths occurred in the 4.5 mg/m³ group, one death occurred in the 1.7 mg/m³ group, and three deaths occurred in the 0.45 mg/m³ group. Six deaths in the female control group were attributed to a defective feeder. Clinical effects included listlessness in the 4.5 mg/m³ and 11 mg/m³ groups. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were reported in exposed males or females. Males in the 0.45 mg/m³ group exhibited a statistically significant decrease in weight, which was not toxicologically significant (i.e., <10%). Body weights of exposed mice were similar to controls in all other groups.

In both rats and mice some statistically significant hematological changes in red blood cell parameters occurred. Although these changes were not dose-related, they are consistent with an adaptive response to impairment of pulmonary gas exchange and add to the weight of evidence that the respiratory system is the major target. Clark et al. (1982) and Rand et al. (1982a) also noted hematological effects in subchronic studies.

As evidenced by a somewhat lower frequency of effects, mice were not as sensitive to the respiratory toxicity of HCCPD as were rats. Male mice exhibited significant increases in suppurative inflammation of the nose and squamous metaplasia of the trachea at 4.5 and 11 mg/m³, and acute necrosis and suppurative inflammation of the nose, acute necrosis of the larynx, trachea, and lung, and congestion of the lung at 22 mg/m³. Female mice had serous inflammation of the nose at 4.5 mg/m³, and suppurative inflammation of the nose, squamous metaplasia of the larynx and trachea, and necrotizing inflammation of the lung at 11 mg/m³. At the highest dose, female mice presented the same spectrum of effects as male mice. Because no effects were observed in mice at 1.7 mg/m³, the NOAEL was 1.7 mg/m³ and the LOAEL was 4.5 mg/m³.

4.2.1.2. *Rand, GM; Nees, PO; Calo, CJ; et al. (1982a) Effects of inhalation exposure to hexachlorocyclopentadiene on rats and monkeys. J Toxicol Environ Health 9:743-760.*

Rand, GM; Nees, PO; Calo, CJ; et al. (1982b) The Clara cell: an electron microscopy examination of the terminal bronchioles of rats and monkeys following inhalation of hexachlorocyclopentadiene. J Toxicol Environ Health 10:59-72.

In these studies, Sprague-Dawley rats and cynomolgus monkeys inhaled, via whole-body exposure, 97.7% pure HCCPD at 0, 0.01, 0.05, or 0.20 ppm (0, 0.11, 0.56, or 2.3 mg/m³,

respectively)² for 6 hours/day, 5 days/week, for 14 weeks. Each exposure group contained 40 male and 40 female rats, or 6 male and 6 female monkeys. To investigate the Clara cell of the lung as a potential target for HCCPD toxicity, Rand et al. (1982b) performed electron microscopy upon lung cell preparations from three rats of each sex and three monkeys of each sex.

Rand et al. (1982a) reported no mortalities or adverse clinical signs in monkeys at any exposure level. Body weight gain and food consumption were not significantly different between groups. Pulmonary function tests (blood gas analysis, lung mechanics, lung ventilation) were normal. No eye lesions were noted, and no exposure-related changes were noted in hematology, clinical chemistry, urinalysis, organ weights, macroscopic pathology, or histopathology. One male monkey from the 2.3 mg/m³ group exhibited occasional Clara cells containing “electron-lucent inclusions in the apex and base of the cell, surrounded by a single limiting membrane.” The electron-lucent inclusions have no known relationship to pathology, so the existence of the inclusions in the Clara cells was not considered to be adverse. As no adverse effects were noted, the NOAEL for monkeys was 2.3 mg/m³ HCCPD.

Rand et al. (1982a) reported that four rats from three exposure groups, including the control group, died or were killed because of severe illness, but illness was not attributed to HCCPD exposure. The only significant clinical sign reported in male rats was dark, red eyes observed in the 0.56 and 2.3 mg/m³ dose groups. This effect, which was first noted after the 10th exposure and disappeared after the 20th exposure, was also noted in a range-finding study performed by the same authors, and was considered to be related to HCCPD exposure. Ophthalmoscopic examination revealed no eye lesions. There were no exposure-related changes in body weight gain, food or water consumption, or urinalysis. After 12 weeks of exposure, there were slight, occasionally statistically significant increases in hemoglobin, red blood cell count, and mean corpuscular hemoglobin concentration with a corresponding reduction in the mean cell volume in males at 0.11 and 2.3 mg/m³ and in females at 0.56 and 2.3 mg/m³. The authors observed similar effects in a range-finding study and considered them to be indicative of impaired respiratory function. There were no other effects on hematology. Statistically significant decreases in mean liver weight occurred in all treatment groups and in kidneys of all treated males after 13 weeks of exposure. The Clara cells of all treated rats contained a statistically significant increase in the number of the electron-lucent inclusions as compared to controls (Rand et al., 1982b). No treatment-related gross pathology or histopathology was observed. Given that the changes in hematologic parameters were not dose-related, the kidney and liver weight changes were not accompanied by pathology, and the Clara cell inclusions were not related to pathology, the NOAEL for rats was 2.3 mg/m³. There was no LOAEL.

²Calculated using conversion of 1 ppm = 11.3 mg/m³.

4.2.1.3. Clark, DG; Pilcher, A; Blair, D; et al. (1982) Thirty week chronic inhalation study of hexachlorocyclopentadiene (HEX) in rats. Group Research Report SBGR.82.051. NTIS/OTIS43022.

In a 30-week study, Wistar rats inhaled 0, 0.05, 0.1, or 0.5 ppm (0, 0.56, 1.1, or 5.6 mg/m³, respectively)³ HCCPD in inhalation chambers for 6 hours/day, 5 days/week, followed by a recovery period of 14 weeks free from exposure. Chemical purity of the compound decreased from 96% to 90% during the course of the study because of oxidation. Clinical signs included sneezing and lethargy in animals exposed to 5.6 mg/m³ throughout the study. Four males and two females from this group died during exposure. Pathological analyses revealed that the animals that died prematurely had signs of bronchopneumonia. Two of the deceased rats had enlarged adrenals and the thorax contained watery or bloodstained fluid. No deaths or clinical signs of toxicity were reported in the other exposure groups.

Males in the 1.1 mg/m³ and 5.6 mg/m³ groups had significantly higher mean erythrocyte counts, hemoglobin concentrations, hematocrit and absolute numbers of neutrophils, and significantly lower lymphocyte counts than the controls. Mean absolute numbers of lymphocytes were lower in females at the 5.6 mg/m³ dose.

Body weights of males from the 5.6 mg/m³ dose group were significantly lower than controls from the seventh week until the end of the study, but, at 6% less than controls, were not toxicologically significant. Several increases in body weights in females exposed to HCCPD, compared to controls, were noted in the first half of exposure. At the end of the exposure period, body weights of the 1.1 mg/m³ and 5.6 mg/m³ females were similar to controls; however, at the end of the recovery period, body weights of those groups were less than controls by 11% and 9%, respectively. Kidney weights were significantly increased in females in the 5.6 mg/m³ group after exposure for 30 weeks. Male heart weights were decreased at 30 weeks in the 5.6 mg/m³ group and male spleen weights were decreased at 44 weeks in the 0.56 and 1.1 mg/m³ groups. Testes weights were significantly increased at 44 weeks in the 5.6 mg/m³ group. The organ weight effects were not considered to be biologically significant by the study authors.

Rats at the 5.6 mg/m³ dose showed pulmonary degenerative changes including epithelial hyperplasia, edema, and sloughing of the bronchiolar epithelium in both sexes and epithelial ulceration and necrosis in the males. No degenerative changes in the lungs were observed in the 0.56 or 1.1 mg/m³ dose groups. Rats in the 5.6 mg/m³ group also had mild degenerative changes in the liver and kidney. The authors suggested that the toxic action of HCCPD involves an extreme local irritation of the respiratory tract that causes death by respiratory failure following bronchopneumonia. The authors considered that the mild degenerative changes in the livers and kidneys of a few rats were unlikely to contribute significantly to HCCPD's toxicity in the rat. The results of this study indicate a NOAEL of 1.1 mg/m³ and a LOAEL of 5.6 mg/m³ for the

³Calculated using conversion of 1 ppm = 11.3 mg/m³.

critical effect of respiratory tract histopathology. Correction for the HCCPD content of the administered compound (90%) gives a NOAEL of 1 mg/m³ and a LOAEL of 5 mg/m³.

4.2.1.4. NTP. (1994) Toxicology and carcinogenesis studies of hexachlorocyclopentadiene in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program Technical Report Series 437:318.

The National Toxicology Program conducted 2-year inhalation exposure studies in F344/N rats and B6C3F1 mice. Groups of 60 animals per sex per species were exposed for 5 days per week, 6 hours per day, to atmospheres containing 0, 0.01, 0.05, or 0.2 ppm (0, 0.11, 0.56, or 2.23 mg/m³, respectively) HCCPD. Ten male and 10 female rats and mice from each exposure group were evaluated at 15 months. Standard bioassay data including body weights, urinalysis, organ weights, pathology, and histopathology were collected. Monitoring of the stability of the compound throughout the study showed that no degradation took place for up to 2 years.

Exposure to HCCPD did not significantly affect survival of rats or mice, but the decrease in survival of female mice approached statistical significance in the 2.23 mg/m³ group owing to suppurative inflammation of the ovary. Body weights of rats were unchanged by HCCPD exposure, but body weights of male and female mice were reduced in the 2.23 mg/m³ group.

Neoplastic lesions: No exposure-related increases in neoplasms were seen in male or female rats or mice. Male rats in the 2.23 mg/m³ group, however, exhibited a significant increase in the incidence of pars distalis adenoma of the pituitary (66%). Because the historical control incidence of pars distalis adenoma in male F344/N rats from other NTP inhalation studies was 60%, NTP considered this tumor to be unrelated to HCCPD exposure. NTP concluded that HCCPD exhibited “no evidence of carcinogenic activity” (NTP, 1994).

Nonneoplastic lesions: In female rats, significant increases in incidence of squamous metaplasia of the larynx were seen in the 0.11 and 2.23 mg/m³ groups, but not in the 0.56 mg/m³ group (see Table 2 for incidence). The lesion, described as stratified squamous epithelium several cell layers thick in areas usually lined by columnar epithelium, was considered to be of minimal severity in all groups. Because there is individual variation in the location of the transition between squamous and columnar epithelium and in obtaining consistent tissue sections in the treated rats, NTP indicated that the significance of this metaplasia is unknown. In addition, a dose-response relationship was not evident. Exposure-related increases in yellow-brown granular pigmentation within the cytoplasm of epithelial cells of the nose, trachea, and lung were also observed in both sexes of rats.

Exposure-related increases in pigmentation of the respiratory epithelium of the nose, trachea, and lung were also seen in male and female mice (see Table 3 for incidence). Female mice also exhibited a dose-related increase in the incidence of suppurative ovarian inflammation that was significantly different from controls at 0.56 and 2.23 mg/m³ HCCPD. At 2.23 mg/m³ HCCPD, increases in suppurative inflammation of the nose were noted in both male and female

Table 2. Incidence^a of selected respiratory tract lesions in rats from NTP (1994)

Lesion	Males				Females			
	0 mg/m ³	0.11 mg/m ³	0.56 mg/m ³	2.23 mg/m ³	0 mg/m ³	0.11 mg/m ³	0.56 mg/m ³	2.23 mg/m ³
Nose pigmentation	1/48	46/50	48/49	48/50	0/50	34/50	47/49	48/50
Trachea pigmentation	0/48	0/50	0/48	5/50	0/50	0/50	0/49	1/50
Lung pigmentation								
Bronchiole	0/50	0/50	0/50	49/50	0/50	25/50	42/49	50/50
Peribronchiole	0/50	0/50	2/50	16/50	3/50	1/50	4/50	27/50
Squamous metaplasia of larynx	NR	NR	NR	NR	9/50	20/50	15/48	24/50

^a Compared with number examined.

NR-not reported.

Table 3. Incidence^a of selected respiratory tract lesions in mice from NTP (1994)

Lesion	Males				Females			
	0 mg/m ³	0.11 mg/m ³	0.56 mg/m ³	2.23 mg/m ³	0 mg/m ³	0.11 mg/m ³	0.56 mg/m ³	2.23 mg/m ³
Nose pigmentation	0/50	45/50	50/50	44/50	0/49	40/50	48/50	41/48
Suppurative inflammation	0/50	0/50	1/50	36/50	4/49	0/50	3/50	40/48
Trachea pigmentation	0/50	29/50	48/50	48/50	0/49	6/50	43/48	42/47
Lung pigmentation	0/49	2/50	42/50	45/50	0/48	0/50	27/50	44/49
Suppurative ovarian inflammation	NA	NA	NA	NA	0/49	3/50	6/50	17/50

^a Compared to number examined.

NA-not applicable.

mice during the interim evaluation at 15 months and at study termination. In the 13-week study, this effect was noted in males at 4.5 mg/m³ and in females at 11 mg/m³ HCCPD.

Necrotizing inflammation of the bronchus/bronchioles, a response observed in NTP's subchronic study, was not reported in the 2-year study in rats or mice. Because rats exhibited no exposure-related pathology or histopathology, the NOAEL for rats was 2.23 mg/m³ HCCPD, the maximum exposure concentration. The NOAEL for mice was 0.56 mg/m³ and the LOAEL was 2.23 mg/m³, based on increased incidence of suppurative inflammation of the nose of both sexes.

Although the suppurative ovarian inflammation is clearly an adverse effect, it is not considered to be the critical effect because its causation by HCCPD exposure is suspect. These lesions were common in NTP studies at the time of the HCCPD study (1984), and have since been reduced through better laboratory practice (Rao et al. 1987). The NTP Pathology Working Group on the HCCPD study did not consider these lesions to be a direct effect of the chemical, but felt they were most likely secondary to stress resulting from exposure. Ovarian abscesses in B6C3F1 mice resulted from bacterial infection, with the bacterium *Klebsiella oxytoca* being isolated most commonly. Dose related increases in ovarian abscesses have been seen in other NTP chronic studies and the reason for this apparent treatment-related effect has been unclear. It has been suggested that stress related to exposure may depress the immune system allowing infection by opportunistic bacteria. The ovarian inflammation is unlikely to have been mouse grouping related, as the mice were housed separately in this inhalation study.

The yellow-brown pigmentation of the respiratory epithelium was considered to be a marker of exposure rather than a toxic effect because it was not associated with any discernible lesion even after prolonged exposure. It was found in both sexes of both species. The pigmentation occurred in all areas of the respiratory tract at the highest exposure, and as the exposure was reduced, only in the proximal portion of the respiratory tract. NTP (1994) suggested that lipid peroxidation may have produced the pigmentation. Although the designation of this pigmentation as nonadverse conflicts with ATSDR's treatment (ATSDR, 1999), it is entirely consistent with the guidance in the RfC methodology (U.S. EPA, 1994b) which indicates that "enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects" should be ranked low in severity. Furthermore, the guidance states that "effects that may be considered marginal are designated as adverse only to the extent that they are consistent with other structural and functional data suggesting the same toxicity," indicating pigmentation does not qualify as an adverse effect in this situation.

4.2.2. Oral Studies

4.2.2.1. *Abdo, KM; Montgomery, CA; Kluwe, WM; et al. (1984) Toxicity of hexachlorocyclopentadiene: Subchronic (13-week) administration by gavage to F344 rats and B6C3F1 mice. J Appl Toxicol 4:75-81.*

This subchronic study investigated the systemic toxicity of HCCPD given by gavage to weanling F344 rats and B6C3F1 mice. HCCPD (97.4% pure) was dissolved in corn oil and

administered daily, 5 days per week for 13 weeks. Ten rats/sex/dose received 0, 10, 19, 38, 75, or 150 mg/kg HCCPD. Ten mice/sex/dose received 0, 19, 38, 75, 150, or 300 mg/kg HCCPD. Stability of the gavage mixture, or the frequency of preparation, was not reported. Although data on clinical signs, body weights, organ weights, gross pathology, and histopathology were collected, no clinical chemistry, hematology, or urine analysis was performed as required by current test guidelines (U.S. EPA, 1998b).

Table 4 shows the mortality rates for rats and mice. The deaths of six male rats in the 150 mg/kg group, and one in the 75 mg/kg group, were attributed to HCCPD. All male mice and three females in the 300 mg/kg group died before the end of the study. Other premature deaths in treated rodents were attributed to gavage error. Clinical signs of ruffled fur and slight inactivity were noted in both rats and mice in the two highest dose groups. Significant body weight decreases (i.e., $\geq 10\%$ less than controls) were noted in male rats in the 38, 75, and 150 mg/kg groups and in female rats in the 75 and 150 mg/kg groups. In mice, significant decreases in body weight were noted in males in the 150 mg/kg group and in females in the 300 mg/kg group. Data from organ weight ratios were significantly greater than controls for female rats at 75 and 150 mg/kg for right kidney:brain and at 38, 75, and 150 mg/kg for liver:brain. Liver:brain and right kidney:brain weight ratios were significantly increased compared to controls at all doses in female mice. In addition, the lungs:brain ratio was significantly elevated over controls at the highest dose in female mice. Organ weight ratios were unaffected in male mice.

Necropsy revealed grossly observed lesions detected in the gastric mucosa in both rats and mice. These lesions consisted of black discolored foci, red cysts, and ulceration in rats gavaged with 75 and 150 mg/kg HCCPD. Thickening of the mucosa was also observed in mice in the 150 and 300 mg/kg groups. Histopathological analyses noted forestomach lesions that ranged from minimal to marked in severity and were focal to diffuse in distribution. Notable features were hyperplasia, acanthosis, and hyperkeratosis of the epithelial surface of the forestomach and increased mitotic activity in the basal layer of the epithelium. Forestomach lesions were only discernible at and above the 38 mg/kg dose in male rats, but were seen (identified as epithelial hyperplasia and focal inflammation) in female rats at the 19 mg/kg dose (see Table 5). Forestomach lesions were noted in male and female mice at the 38 mg/kg dose

Table 4. Mortality for mice and rats (Abdo et al., 1984)

Dose (mg/kg)	Male rats	Female rats	Male mice	Female mice
0	3/10	1/10	1/10	0/10
10	1/10	2/10	-	-
19	1/10	2/10	0/10	0/10
38	1/10	1/10	0/10	0/10

75	3/10	3/10	0/10	0/10
150	7/10	5/10	0/10	0/10
300	-	-	10/10	3/10

Table 5. Incidence^a of stomach and kidney lesions in rats from Abdo et al. (1984)

Dose (mg/kg) Lesion	Males						Females					
	0	10	19	38	75	150	0	10	19	38	75	150
Stomach lesions	0/10	0/10	0/10	5/10	9/10	8/9	0/10	0/10	2/10	5/10	9/10	9/10
Toxic nephrosis	0/10	0/10	0/10	10/10	9/10	8/10	0/10	0/10	0/10	10/10	10/10	10/10

^a Compared to total number of animals examined.

(see Table 6). The forestomach lesions are believed to be a manifestation of irritation which is consistent with the observation of dermal irritation, (Treon et al., 1955; Industrial Bio-test Laboratories, 1975a; HEW, 1978) and other portal-of-entry effects from HCCPD exposure (Clark et al., 1982; NTP, 1994). No forestomach lesions were observed in control rodents of either species.

Toxic nephrosis of the kidney was observed in male and female rats in the 38, 75, and 150 mg/kg groups, and in female mice in the 75, 150, and 300 mg/kg groups (see Tables 5 and 6, respectively). The tables show that the incidence of this response was zero at the two lower doses and approximately maximal at the higher doses. This dose-response pattern may reflect the steepness of the dose-response curve. The kidney lesions were predominantly limited to the terminal portion of the proximal convoluted tubules in the inner cortex and were characterized by dilated tubules and epithelial changes consisting of cytomegaly, karyomegaly, and anisokaryosis with nuclear and cytoplasmic vacuolization. Acute tubular necrosis, which was morphologically distinct from the toxic nephrosis, was observed in 7 of the 10 male mice in the 300 mg/kg group and may have caused the early mortality in this group. Although histopathologic changes in mice did not occur at doses below 38 mg/kg HCCPD, liver weights increased in a dose-dependent fashion starting at 19 mg/kg HCCPD. Because organ weight changes occurred only in females of both rodent species, and toxic nephrosis was not observed in male mice, this report indicates that female rodents may be generally more susceptible to the adverse effects of ingested HCCPD to the kidney and liver.

Table 6. Incidence^a of stomach and kidney lesions in mice from Abdo et al. (1984)

	Males						Females					
Dose (mg/kg) Lesion	0	19	38	75	150	300	0	19	38	75	150	300
Stomach lesions	0/10	0/10	2/10	8/10	9/10	10/10	0/10	0/10	2/9	9/10	10/10	9/9
Toxic nephrosis	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	9/10	10/10	7/10

^aCompared to total number of animals examined.

Based on the irritant effect manifested by the incidence of forestomach lesions, the NOAEL for both sexes of mice was 19 mg/kg. The LOAEL was 38 mg/kg HCCPD. For rats, the NOAEL was 10 mg/kg based on the incidence of forestomach lesions in female rats. The LOAEL for rats was 19 mg/kg.

4.2.2.2. *Industrial Bio-test Laboratories. (1975b) 90-Day subacute oral toxicity study with C-56 in albino rats. Unpublished report to Hooker Chemical Corporation. Doc # 878212102. NTIS/OTS84003A.*

In this study, 0, 30, 100, and 300 ppm HCCPD of unknown purity was fed to 15 weanling male and 15 female Charles River rats per group. The diet was prepared by preblending the required amount of HCCPD with the chow in a high-speed blender. Fresh diets were prepared on a weekly basis. No precautions to prevent degradation of the test compound during diet preparation or throughout the study were reported. During the 90-day study, animal weights, food consumption, and clinical signs were recorded. Blood chemistry, hematology, and urinalyses were analyzed at 45 and 84 days. Animals were sacrificed after 90 days, at which time gross examinations, organ weight comparisons, and microscopic examinations were performed.

The authors reported no statistically significant differences between exposed and control populations that were related to HCCPD exposure. On day 45 total leukocyte counts in males and females at 300 ppm were statistically lower than controls (rats at the lower doses were not tested). On day 84, however, male rats at 30 and 100 ppm, and female rats at 100 ppm had statistically higher total leukocyte counts than controls, whereas total leukocyte counts in both sexes at 300 ppm were not different from controls. Thus, the response did not follow a consistent dose-response pattern and may be unassociated with HCCPD exposure. Statistical differences in hemoglobin concentration followed the same pattern of dose and duration as those for total leukocyte count. The authors indicated that even though some of the hematologic changes in treated animals were statistically different from controls, the values were still within the limits of normal variation. All other measured parameters, including food consumption, body weight gain, organ weights, hematology, clinical blood chemistry, and urinalyses revealed no exposure-related differences between control and exposed populations.

The results of this study identify a NOAEL of 300 ppm HCCPD in food for male and female rats. Multiplying the total food consumed by the amount of HCCPD in food (i.e., 300 mg HCCPD/kg) and dividing by the number of days on the study (i.e., 90 days) yielded an average daily consumption of 6.9 mg HCCPD/day for males and 5.0 mg HCCPD/day for females. Dividing the average daily consumption of HCCPD by the average weight of the animals yielded NOAEL doses of 21.4 mg/kg/day for males and 25 mg/kg/day for females. However, as the HCCPD was not tested for degradation throughout the study and the HCCPD/food mixture was prepared only on a weekly basis, the stability of the test compound is in question. The absence of observable effects in this study could be a direct result of the degradation of the compound from exposure to light after diet preparation. Additional misgivings about this study are due to the fact that it was performed during a time when critical

errors were committed at Industrial Bio-test Laboratories. Although they may not have occurred in this particular study, any data from this period are suspect.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

There are no animal studies available on developmental or reproductive effects of HCCPD after inhalation exposure. The following studies suggest a lack of developmental effects following oral exposure, although degradation of the highly photoreactive HCCPD may have occurred in some studies.

4.3.1. Murray, FJ; Schwetz, BA; Balmer, MF; et al. (1980) Teratogenic potential of hexachlorocyclopentadiene in mice and rabbits. *Toxicol Appl Pharmacol* 53: 497-500.

HCCPD was tested for teratogenicity by administration to an unspecified number of pregnant CF-1 mice and New Zealand white rabbits via oral gavage in cottonseed oil on gestation days 6–15 for mice or 6–18 for rabbits. The test doses were 0, 5, 25, or 75 mg/kg. Mice were sacrificed at gestation day 18 and rabbits were sacrificed at gestation day 29. Gas chromatography indicated the HCCPD preparation was stable for at least 7 days.

No significant effects were seen for number of implantations, fetus viability, resorptions, or mean fetal body measurements. Maternal toxicity in the form of severe diarrhea and subsequent death in an unspecified number of rabbits was seen at 75 mg/kg. A dose-related increase in the proportion of rabbit fetuses with 13 ribs was seen and was statistically significant in the 75 mg/kg group. Given the authors' statement that 12 or 13 ribs in this species is normal, this increase is not considered to be a significant effect. No other dose-related effects on incidence of fetal malformations in mice or rabbits were seen. The authors concluded that HCCPD was not teratogenic in mice or rabbits at the doses given.

4.3.2. Chernoff, N; Kavlock, RJ. (1983) A teratology test system which utilizes postnatal growth and viability in the mouse. *Environ Sci Res* 27:417-427.

The teratogenicity of HCCPD was tested in mice using a simple screening procedure based on the assumption that prenatal effects would be manifested as changes in two easily measured postnatal parameters (pup viability and growth). This assay was performed with a number of chemicals and found to predict the results of standard, more labor-intensive teratogenicity tests with sufficient accuracy. Twenty-five pregnant CD-1 mice were gavaged with 45 mg/kg HCCPD on gestation days 8–12, the period of major organogenesis. Gestation was allowed to continue until delivery at day 19.

No significant differences in maternal weight change, pup survivorship, or average pup weight were seen between treated animals and untreated controls. The authors' conclusion that HCCPD was not a teratogen under the conditions of this assay agrees with the results of the standard mouse assay in Murray et al. (1980).

4.3.3. Goldenthal, EI; Jessup, DC; Rodwell, DE. (1978) Teratology study in rats. Unpublished report by International Research and Development Corporation for Velsicol Chemical Corporation. Report No. 163-573. Doc #40-8249076, NTIS/OTS0512884.

The Velsicol Chemical Corporation performed teratogenicity studies with HCCPD in CD rats (Goldenthal et al., 1978). Groups of 25 pregnant rats were administered doses of 0, 3, 10, or 30 mg/kg HCCPD via corn oil gavage on gestation days 6–15 and were sacrificed on day 20. No significant maternal effects were seen, and no significant fetal effects were seen as measured by mean number of implantations, corpora lutea, live fetuses, postimplantation losses, mean fetal body weights, fetal sex ratios, or incidence of soft-tissue or skeletal malformations. No details were provided on possible precautions taken to prevent compound degradation during the experiment.

4.4. OTHER STUDIES

4.4.1. Contact Dermatitis

Several studies have evaluated the dermal toxicity of HCCPD in rabbits and guinea pigs. A preliminary study involved painting 300 mg/kg HCCPD on the skin (location unspecified) and sacrificing the animal after 24 hours (HEW, 1978). Gross pathology revealed subcutaneous edema from the inguinal region to the mediastinal area. Rib impressions on the parietal surface were apparent from expanded lungs. Histopathology of the lungs revealed atelectasis with thickened alveolar walls containing moderate numbers of macrophages and neutrophils. Histopathology of the skin revealed that the squamous epithelium was one cell thick. No hyperkeratosis or mitotic activity or necrosis of epithelial cells was apparent. Collagen bundles were disrupted by moderate edema and focal pockets of neutrophils were seen in the dermis. Both the dermis and the adipose tissue layer were edematous.

A second preliminary study using doses of 0, 300, 600, and 1,200 mg/kg painted on the skin (location unreported) of one guinea pig/dose resulted in adverse effects similar to those observed in acute oral studies in which rats had been administered up to 300 mg/kg HCCPD in corn oil via gavage. These effects included sneezing, erythema of the eyelids and ears, rhinitis, cyanosis of the lips and feet, retraction of the head, and labored breathing. In addition, the guinea pigs had black, crusty lesions at the point of HCCPD application (HEW, 1978). The animal dosed with 1,200 mg/kg died 6 hours after treatment.

Treon et al. (1955) applied various solutions of 93.3% HCCPD in Ultrasene to the intact skin of a monkey and two guinea pigs to determine the concentration that produced dermal irritation. When applied to the back of the monkey, 0.05 mL of the 20% solution discolored the skin immediately. After five days, the skin was slightly swollen and after 12 days the skin was scaly. The 10% solution applied to the abdomen produced no signs of irritation. Thus, the threshold concentration for producing dermal irritation in monkeys is between 10% and 20% HCCPD. When applied to the back of a guinea pig, solutions of HCCPD up to 1% produced no

effects. On another guinea pig, the lowest concentration tested that produced an effect was 40%. The skin became hard, encrusted, and necrotic. Thus, the threshold concentration for irritating the skin of guinea pigs is between 1% and 40% HCCPD.

A 28-day dermal toxicity test was performed using 0.1% and 0.5% HCCPD (w/v) dissolved in denatured ethyl alcohol (Industrial Bio-test Laboratories, 1975a). The solutions were applied 5 days/week for 4 weeks to the shaved skin of five female and five male rabbits. These doses were equivalent to 1 mg/kg and 5 mg/kg, respectively. The skin of two males and two females in each group was abraded. After the first application, a slight red erythema was noticeable. After the seventh application, focal necrosis, escharosis, hemorrhaged fissures, and pustules with odorous exudate were reported in both dose groups. Slight-to-moderate (1 mg/kg) or moderate-to-severe (5 mg/kg) desquamation was observed after 20 applications. No deaths occurred, and although a few of the animals in the high-dose group lost weight at 14 days (corresponding to the severity of the skin reactions), the animals regained the weight as the lesions healed and formed scabs and scars. No treatment-related effects were reported on hematology, blood chemistry, urinalyses, or gross or microscopic pathology tests.

4.4.2. Genotoxicity

A battery of in vitro and in vivo genotoxicity studies performed by the National Toxicology Program yielded generally negative results for HCCPD (NTP, 1994). Absence of mutagenicity observed in Ames reversion assays using *Salmonella typhimurium* (*S. typhimurium*) strains TA98, TA100, TA1535, and TA1537, with or without S9 fraction confirmed earlier results by Industrial Bio-test Laboratories (1977) and Brooks et al. (1984). NTP (1994) also obtained negative results for micronucleated erythrocyte frequency in the B6C3F1 mice exposed to HCCPD for 13 weeks by inhalation, and for induction of sex-linked recessive lethal mutations in male *Drosophila melanogaster*. The negative results in *Drosophila melanogaster* essentially duplicated earlier analyses (Zimmering et al., 1985; Mason et al., 1992). When administered to male flies at 10–40 mg/kg in feeding solutions, or at 900–2,000 mg/kg by injection, HCCPD did not increase the number of lethal mutations in male *Drosophila* when compared to controls. However, cytogenetic effects manifested as sister chromatid exchanges and chromosomal aberrations were observed in Chinese hamster ovary cells exposed to HCCPD, with and without S9 (NTP, 1994).

Brooks et al. (1984) used a preincubation protocol suitable for volatile chemicals to incubate five strains of *S. typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100) with HCCPD at concentrations up to 10 µg/mL (37 µM) in the absence of S9 fractions, or 500 µg/mL (1.8 mM) in the presence of S9 fractions. There was no evidence of mutagenesis. Similar results were obtained when *S. typhimurium* strain TA100 was incubated for 30, 60, or 120 minutes in the presence of HCCPD as a volatilate at 500–2,500 µg/mL (183 mM–917 mM; Industrial Bio-test Laboratories, 1977). As the exposure duration was increased over 120 minutes, cell survival decreased at each concentration tested, indicating that HCCPD is cytotoxic in this concentration range. HCCPD did not induce chromosome damage in metaphase stage rat liver (RL4) cells after a 24-hour incubation at 0.2 µg/mL (0.8 µM), the highest nontoxic concentration tested (Brooks et

al., 1984). HCCPD did not induce a significant increase in morphological transformation in BALB/3T3 cells (at concentrations up to 0.000156 μL technical grade HCCPD/mL incubation medium, or $1.6 \times 10^{-5}\%$) and did not induce forward mutations in mouse lymphoma cells at non-cytotoxic concentrations (up to 0.00125 μL technical grade HCCPD/mL incubation medium, or $1.3 \times 10^{-4}\%$) (Litton, 1978). HCCPD at subtoxic concentrations also did not induce DNA repair when incubated with rat hepatocytes in vitro (Brat, 1983).

4.4.3. Acute Toxicity

The acute toxicity of HCCPD via inhalation and oral exposure is well established. Treon et al. (1955) performed the only published study for these exposure routes in several different animal species. The lethal dose of a 93.3% pure solution of HCCPD (5% V/V in peanut oil) administered via gavage to female rabbits ranged between 420 and 620 mg/kg. The authors also administered the same solution of HCCPD at doses of 180 to 2,100 mg/kg to groups of 10 six-month-old rats per dose. The numbers of deaths and adverse effects were recorded for 10 days. The LD_{50} for male rats was 505 mg/kg. Rats and rabbits that died exhibited diffuse degenerative changes in the brain, heart, liver, and adrenal glands; degeneration of the liver, and kidney tubules, and pulmonary hyperemia and edema. An earlier study using Spartan albino rats administered HCCPD in corn oil at 10 mL/kg body weight (Wazeter and Geil, 1972). The results yielded a LD_{50} of 630 mg/kg for males and 530 mg/kg for females, with a combined LD_{50} for both sexes of 584 mg/kg. The purity of the HCCPD was not reported for this study.

Industrial Bio-test Laboratories (1975c) investigated the acute inhalation toxicity for HCCPD (unreported purity) using groups of five male and five female Charles River rats exposed to 2.5 to 21 ppm (28.2–237 mg/m^3)⁴ HCCPD for 4 hours. The LC_{50} was estimated as 38.4 mg/m^3 . Necropsies performed on animals that died revealed acute pneumonia, with the lungs showing varying degrees of hepatization (i.e., gorged with effused matter so that they are no longer pervious to air). Surviving rats were emaciated and often the lungs did not collapse when the thorax was opened. This phenomenon suggests a chronic proliferative inflammatory response in the lungs.

Wazeter and Geil (1972) also studied acute inhalation toxicity of HCCPD (purity unreported) using two sets of 10 male Carworth CFE rats. The rats inhaled either 2 or 200 mg/L (2,000 or 200,000 mg/m^3 , respectively) HCCPD for 4 hours. All died within 48 hours of exposure. Clinical signs included eye squint, dyspnea, cyanosis, salivation, lacrimation, ocular and nasal porphyrin discharge, and erythema followed by blanching and hypoactivity. Necropsy revealed congestion of the lungs in all rats at the low dose, while rats at the high dose had gray coloring of the skin and severe hemorrhage of the lung and hydrothorax.

Treon et al. (1955) performed acute and subacute inhalation toxicity studies on guinea pigs, rats, mice, and rabbits. The concentrations ranged from 1.7 mg/m^3 (89.5% HCCPD) to 804

⁴Calculated using conversion of 1 ppm = 11.3 mg/m^3 .

mg/m³. The duration of exposure was increased in some experiments with lower doses (e.g., 3.6 mg/m³ was administered five times with each exposure lasting 7 hours). Clinical signs and fatalities were recorded. LC₅₀s were not estimated. A concentration of 143 mg/m³ for 3 hours resulted in fatalities among rabbits, rats, and mice, but not among guinea pigs. The authors noted that rabbits appeared to be the most susceptible species, with mice, rats, and guinea pigs exhibiting decreasing susceptibility, in that order. Exposure to concentrations as low as 3.6 mg/m³ irritated the eyelids and increased respiratory rate after 2 or 3 days (species not indicated). Prolonged intermittent exposure (150 exposures of 7 hours each) to 1.7 mg/m³ HCCPD, the lowest concentration administered, resulted in slight degenerative changes in the livers and kidneys of all species observed. Mice exhibited pulmonary edema and bronchitis, and some of the guinea pigs and rats developed pneumonia (incidence not specified). Rabbits did not appear to manifest an inflammatory response at 1.7 mg/m³.

Ulrich and Hagan (1978) administered HCCPD (unknown purity) at 8 different concentrations from 0.28 to 5.8 ppm (3.2 to 66 mg/m³)⁵ to groups of 10 male and 10 female Sprague-Dawley rats. The experiment consisted of inhalation exposure to HCCPD for 4 hours, followed by a 14-day observation period. The 4-hour LC₅₀ was 18 mg/m³ for male rats and 41.3 mg/m³ for females, which indicated that males are more sensitive to the compound. The LC₅₀ for females was similar to the 38.4 mg/m³ LC₅₀ calculated by Industrial Bio-test Laboratories (1975c) using both sexes. Ulrich and Hagan (1978) observed some degree of sedation in all rats exposed to 16 mg/m³ or greater, and dyspnea in all animals at 40 mg/m³ or greater. Tearing, salivation, and ataxia were observed in most animals exposed to 66 mg/m³. All animals in the 3.2 mg/m³ group gained weight normally over the 14-day observation period while animals in all other exposure groups (16–66 mg/m³) lost weight. Necropsies indicated that animals exposed to 16 mg/m³ or greater had red focal or diffuse consolidation of the lungs progressing to severe generalized hemorrhage and hepatization that was dose-dependent. Some animals in the 66 mg/m³ group also had rhinorrhea and mottling of the liver. The authors noted that despite the biphasic mortality curve (indicating potentially two toxic responses), only pulmonary abnormalities were found.

These studies indicate that HCCPD vapors are very toxic and cause respiratory effects during repeated exposures to low concentrations such as 1.7 mg/m³. Treon et al. (1955) indicated that the acute inhalation toxicity of HCCPD was greater than that of phosgene.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

There are no epidemiologic data concerning the chronic health effects of HCCPD alone in humans. Mortality studies from several plants at which HCCPD was used cannot distinguish between effects from exposure to HCCPD and effects from exposure to other chlorinated compounds present. The presence of other chemicals, however, is not a confounder because the

⁵Calculated using conversion of 1 ppm = 11.3 mg/m³

studies reported no significant increases in death from any causes, including cancer, for employees exposed to HCCPD and other chlorinated chemicals compared with matched populations from the United States (Brown et al., 1980; Buncher et al., 1980; Shindell, 1980; Shindell, 1981; Wang and MacMahon, 1979).

An occupational study (Boogaard et al., 1993) of the chronic effects of HCCPD, which followed more sensitive health measures than the mortality studies, also found no significant health effects. Male chemical plant operators exposed to HCCPD (0.11 mg/m³), allyl chloride (3 mg/m³), 1,3-dichloropropene (<5 mg/m³), and epichlorohydrin (< 4 mg/m³) for an average of 8.2 years did not show any differences in liver and kidney function tests as compared to controls. The data indicate that chronic exposure to this mixture of chlorinated solvents did not cause significant liver or kidney damage under these occupational exposure conditions.

An acute occupational exposure to HCCPD at concentrations that may have been as high as 211 mg/m³ produced eye irritation, headache, persistent fatigue, chest discomfort, skin irritation, and cough that persisted for up to 6 weeks following exposure (Kominsky et al., 1980). Liver function studies on workers detected slight increases in serum glutamate-oxaloacetate transaminase, alkaline phosphatase, total bilirubin, and lactate dehydrogenase. These changes suggest that acute exposure to high concentrations of HCCPD may result in liver damage, but the relationship of HCCPD exposure to hepatotoxicity is confounded by inadequate preexposure monitoring, the presence of OCCP, and the lack of definitive exposure data.

Three developmental toxicity studies showed that oral HCCPD did not induce adverse developmental effects in mice, rats, or rabbits, even at doses that induced severe maternal toxicity such as diarrhea and subsequent death in rabbits (Murray et al., 1980; Chernoff and Kavlock, 1983; Goldenthal et al., 1975). Oral doses as high as 75 mg HCCPD/kg were tested.

The metabolic pathways of HCCPD are not well known. Pharmacokinetic studies in mice, rats, and rabbits indicate that absorption, distribution, and excretion of HCCPD depend on exposure route. Orally administered HCCPD is poorly absorbed (Mehendale, 1977; Yu and Atallah, 1981; Lawrence and Dorough, 1981, 1982). Although the relative concentration varies with route, the kidneys, liver, and lungs are the predominant sites for HCCPD distribution. Oral HCCPD concentrates mainly in the kidneys, followed by the liver and then the lung (Lawrence and Dorough, 1981, 1982). Distribution studies involving both rats (Lawrence and Dorough, 1981, 1982) and mice (Dorough and Ranieri, 1984) indicate that inhaled HCCPD deposits primarily in the trachea, followed by the lungs and the kidneys. IV HCCPD deposits in the kidneys, followed by the lungs and then the liver. The exposure route also influences the excretion of HCCPD. Inhaled HCCPD is excreted primarily in the urine, whereas oral HCCPD is excreted mainly via the feces. The larger proportion of excretion via feces after oral administration is due, at least partly, to the larger proportion of biliary excretion. Approximately equal proportions of an IV dose end up in urine and feces. Metabolism of radiolabeled HCCPD in rodents is rapid, with the majority of the radiolabel excreted within 24 hours of administration (Yu and Atallah, 1981; Lawrence and Dorough, 1981, 1982; Dorough and Ranieri, 1984).

Attempts to characterize the polar metabolites from tissue homogenates or urine or fecal samples have been unsuccessful (Mehendale, 1977; Yu and Atallah, 1981; Logan and Croucher, 1984).

4.5.1. Inhalation Studies

There are several subchronic inhalation toxicity studies available as well as one study of chronic duration. Although no adverse effects were noted in monkeys or rats exposed to up to 2.23 mg/m³ HCCPD in a subchronic regimen, rats exhibited minor changes in hematologic parameters, which were not dose-related, after 12 weeks of exposures as low as 0.11 mg/m³ (Rand et al., 1982a). In another subchronic study, Clark et al. (1982) identified the lungs as a target organ for HCCPD toxicity. Four of 20 rats exposed to 5.5 mg/m³ HCCPD died from bronchopneumonia. That exposure also produced epithelial hyperplasia, edema, sloughing of bronchiolar epithelium, and epithelial ulceration and necrosis. Decreases in body weight were noted at 1.1 mg/m³. Changes in hematologic parameters with no consistent dose or duration relationship were also noted. A later subchronic study using rats and mice also found hematologic changes in no dose-related pattern and confirmed the respiratory tract pathology (NTP, 1994). Necrotic and suppurative inflammation of the lung occurred in male rats exposed to 4.5 mg/m³ HCCPD. Higher exposures, 11 and 22 mg/m³, produced more severe lesions such as extensive coagulation necrosis in the epithelium of the respiratory tract, inflammatory signs, and 100% mortality. Mortality (3/20) was observed in mice exposed to doses as low as 0.45 mg/m³ in the absence of respiratory tract histopathology (NTP, 1994). The 2-year NTP (1994) study found no respiratory tract pathology in rats exposed to up to 2.3 mg/m³ HCCPD or in male or female mice exposed to up to 0.56 mg/m³. At 2.3 mg/m³, mice exhibited suppurative inflammation of the nose. A dose-related increase in the incidence of suppurative ovarian inflammation was seen in female mice, but it was not considered to be the critical effect because it was a common occurrence attributed to laboratory management procedures (Rao et al., 1987). Neither rats nor mice showed any evidence of exposure-related carcinogenicity.

4.5.2. Oral Studies

Only subchronic studies are available for the oral route of exposure. HCCPD administered via gavage for 13 weeks was responsible for rat mortality at doses as low as 75 mg/kg and mouse mortality at 300 mg/kg (Abdo et al., 1984). Forestomach lesions were observed at 19 mg/kg in female rats and at 38 mg/kg in male rats and both sexes of mice (Abdo et al., 1984). Toxic nephrosis was seen at 38 mg/kg in both sexes of rats and at 75 mg/kg in female mice. Although they did not develop toxic nephrosis at any dose, male mice developed acute tubular necrosis at 300 mg/kg. The other major toxic effect in this study was significantly reduced body weight beginning at 38 mg/kg in rats and 150 mg/kg in mice. No adverse effects were noted at 19 mg/kg in mice or at 10 mg/kg in rats. HCCPD administered in feed for 90 days produced no effects in rats at doses of up to 21-25 mg/kg/day (Industrial Bio-test Laboratories, 1975). The actual delivered dose in this study is questionable, however, because the stability of HCCPD in the weekly prepared diet was not verified.

4.5.3. Mode of Action

HCCPD is a highly reactive chemical as evidenced by its portal-of-entry effects. With two double bonds, it is very reactive with mucous membranes and thus highly irritating to the eyes, respiratory system, and stomach. The biological reactivity of HCCPD, a conjugated diene, may be a result of its high reactivity in Diels-Alder reactions, in which it combines with an alkene (a dienophile) in a cycloaddition reaction (ATSDR, 1999). Spontaneous reactions of this type may explain the fecal elimination as well as the portal-of-entry effects. Potential biological reactants with HCCPD include quinones, sterols, 2-alkenoic acids, unsaturated fatty acids, and unsaturated fatty acid derivatives.

An alternative explanation for irritation of mucous membranes is cleavage of the -C-Cl bond by mixed-function oxidases, which yields free radicals (Cl·) that readily attack unsaturated fatty acids in cell membrane lipids and produce lipid peroxidation. The yellow-brown pigmentation in the respiratory epithelium of rodents chronically exposed to HCCPD by inhalation is thought to be a result of lipid peroxidation (NTP, 1994). The kidney and liver are also target organs because of the high activity of biotransformation enzymes such as the mixed function oxidase types. The kidney nephrosis in the subchronic oral study (Abdo, 1984) in rats and mice is localized in the proximal tubule, which has a high activity of enzymes associated with biotransformation of HCCPD and a high capacity to concentrate chemicals intracellularly.

4.6. WEIGHT OF EVIDENCE EVALUATION AND CANCER CLASSIFICATION—SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE; CONCLUSIONS ABOUT HUMAN CARCINOGENICITY AND MODE OF ACTION

The apparent inability of HCCPD to cause genotoxic effects, and the lack of evidence for both human and animal carcinogenicity, justify the conclusion that HCCPD is not likely to present a human cancer risk by the inhalation route of exposure. According to the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), the evaluation of the overall weight of evidence for carcinogenicity to humans indicates that HCCPD is most appropriately characterized as Group E—Evidence of Noncarcinogenicity to Humans by the inhalation route. In accordance with U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), HCCPD is not likely to be a human carcinogen by the inhalation route based on current data indicating no evidence of cancer in well-conducted bioassays in two species of rodents; the absence of increased deaths from cancer in the limited human occupational studies available; and lack of mutagenicity in a variety of test systems. In a well conducted 2-year inhalation bioassay, no increased incidence of tumors was reported in male or female rats and mice up to 2.2 mg/m³ (NTP, 1994). Several occupational epidemiological studies reported no increase in cancer mortality associated with HCCPD exposure, in the presence of other chlorinated production compounds. Mutagenicity studies were negative in five strains of *S. typhimurium*; negative in mouse micronucleus assays; showed no evidence of transformation of BALB/3T3 cells or forward mutations in mouse lymphoma cells; did not induce DNA repair when incubated with rat hepatocytes; and failed to induce lethal mutations in the offspring of male *Drosophila*. The only

positive result for mutagenicity was an isolated statistically significant increase in sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, but chromosome damage did not occur in metaphase stage rat liver cells.

Because the existing chronic health effect data in both humans and animals covers only the inhalation route of exposure, the potential for carcinogenicity by the oral route is indeterminate. Additionally, there are no data on the carcinogenic potential of HCCPD in developing organisms.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

There are no human studies that indicate the relative sensitivity of children and adults to the toxic effects of HCCPD. There are no animal inhalation studies for developmental effects, but oral studies that administered HCCPD during organogenesis showed no significant fetal effects (Chernoff and Kavlock, 1983; Goldenthal et al., 1978) even at doses that cause severe maternal effects (Murray et al., 1980). On the basis of these results, it is unlikely that HCCPD causes developmental effects in humans. In the absence of data on the effects of HCCPD in juvenile animals, its effects in children cannot be predicted.

4.7.2. Possible Sex Differences

Epidemiology studies have not provided adequate information on sex differences in susceptibility to HCCPD toxicity. The mortality studies (Buncher et al., 1980; Wang and MacMahon, 1979; Shindell and Associates, 1980, 1981; Brown et al., 1980) and single occupational cohort (Boogaard et al., 1993) were predominantly limited to men and did not report significant health effects. Subchronic inhalation studies in cynomolgous monkeys reported no sex differences. Several subchronic studies in rodents, however, suggested that female rodents are more sensitive to sublethal effects whereas males are more sensitive to the lethal effects. Abdo et al. (1984) found more male rodents than female rodents died at the higher doses during a subchronic gavage study, but female rats were more sensitive to forestomach lesions than male rats, and female mice were more sensitive to toxic nephrosis than male mice. A subchronic inhalation study generally reported that more male mice than females died at doses producing mortality (NTP, 1994). For both rats and mice, males were more sensitive than females to respiratory tract inflammation (NTP, 1994). In the chronic inhalation study, however, there were no clear differences in the sensitivity of male and female rodents. There are no mechanistic data available to support or refute male-female differences in sensitivity in animals, and thus no way to predict those susceptibilities in humans.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE

5.1.1. Choice of Principal Study and Critical Effect with Rationale and Justification

No chronic oral studies for HCCPD were identified. There were two subchronic oral studies in rodents. One is a gavage study in rats and mice by Abdo et al. (1984) and the other is a dietary study by Industrial Bio-test Laboratories (1975b). Although gavage administration is not ideal for extrapolation to human exposure, there are two main reasons for choosing Abdo et al. (1984) as the principal study: (1) no effects were observed in the Industrial Bio-test Studies, and (2) effects were noted at lower doses than those given in the Industrial Bio-test Laboratories (1975b) study. Although dietary administration is more relevant to human exposure, there are three main reasons not to choose Industrial Bio-test Laboratories (1975b) as the principal study: (1) the quality of the data is suspect because the study was performed during a time when critical errors were committed at Industrial Bio-test Laboratories, and (2) it was not published in the peer-reviewed literature. Neither study reported sufficient information on the degradation of the dosage preparation. Although Industrial Bio-test Laboratories (1975b) prepared the HCCPD-food mixture weekly, stability information was not reported. Because HCCPD degrades when exposed to light (HSDB, 1999), the HCCPD may have degraded while exposed to light in the animal food bins. Abdo et al. (1984) did not report the frequency of dosage preparation or storage conditions. The results of Abdo et al. (1984), however, do confirm that adequate chemical was delivered to produce effects at 14 mg/kg/day (duration-adjusted), whereas Industrial Bio-test Laboratories (1975b) observed no effects at the highest doses tested: 21.4 mg/kg/day in male rats and 25 mg/kg/day in female rats.

Abdo et al. (1984) administered 0, 10, 19, 38, 75, or 150 mg HCCPD/kg in corn oil by gavage 5 days per week for 13 weeks to 10 F344 rats/sex. Ten B6C3F1 mice/sex were administered 0, 19, 38, 75, 150 or 300 mg HCCPD/kg on the same schedule. Mortality, significant decreases in body weight, and forestomach lesions were observed in all rodents at the higher doses. Toxic nephrosis was also reported in male and female rats and in female mice. The toxic nephrosis was characterized by proximal tubular dilation, cytomegaly, karyomegaly, and anisokaryosis with nuclear and cytoplasmic vacuolization and occurred at doses higher than those producing forestomach lesions. The forestomach lesions were characterized in rats by a varying degree of inflammation associated with hyperplasia in the surface epithelium with the formation of vesicles or bullae and ulceration and erosion of the mucosa. Lesions in mice consisted mainly of inflammation and proliferation, with ulceration restricted to the highest dose in both sexes. The forestomach lesions are believed to be a manifestation of chronic irritation, which is consistent with the observation of dermal irritation (Treon et al., 1955; Industrial Bio-test Laboratories, 1975a; HEW, 1978) and other portal-of-entry effects from HCCPD exposure (Clark et al., 1982; Rand et al., 1982a; NTP, 1994).

Because chronic irritation manifested by forestomach pathology was the most sensitive treatment-related adverse effect, it was identified as the critical effect. Rats were more sensitive than mice. Forestomach lesions were observed in female rats beginning at 19 mg/kg and in both sexes of mice beginning at 38 mg/kg. The NOAEL for this lesion in female rats was identified as 10 mg/kg and the LOAEL was 19 mg/kg (see Table 7).

5.1.2. Methods of Analysis—Benchmark Dose Analysis

The incidence of treated animals with stomach lesions is a quantitative measure of toxicity that allows benchmark dose analysis. Benchmark dose modeling was applied to these data because there was a clear increase in response with dose and there were at least two doses that produced more than minimal but less than maximal effects. Only data from female rats were used because this sex was more sensitive to HCCPD toxicity based on the presence of a response in females at 19 mg/kg, which did not produce a response in males (Abdo et al., 1984). The dose-response data and the conversion to continuous dosing are shown in Table 7. Because Abdo et al. (1984) provided gavage administration 5 days per week, the doses were adjusted to daily doses by multiplying by 5 days/week and dividing by 7 days/week. Thus, the duration-adjusted NOAEL and LOAEL are 7 and 14 mg/kg/day, respectively.

Benchmark dose (BMD) analysis was chosen for dose-response analysis because it uses the entire doseresponse curve to identify the point of departure, it does not depend upon dose spacing, and it is sensitive to the number of animals used in the study. The data available met the suggested criteria (U.S. EPA, 1995) of at least three dose levels with two doses eliciting a greater than minimum and less than maximum response. Nine statistical models from U.S. EPA's Benchmark Dose Software (v1.2) were applied to the data to identify the model that best fit the dose-response curve (see Appendix B). The models with good statistical fit, as evidenced by goodness-of-fit p-values >0.05 , were retained for evaluation of the Akaike Information Criterion (AIC), a measure of the deviance of the model fit adjusted for the degrees of freedom, and evaluated for visual fit in the low dose region, which approximates 10% response. The model with the lowest AIC and best visual fit is used to estimate the BMD_{10} (dose predicted to cause a 10% increase in the incidence of the effect) and the $BMDL_{10}$ (the 95% lower confidence limit on the BMD_{10}). Visual ranking is important to assess whether the calculated curve fits well in the 10% response range.

Six of the nine statistical models met the statistical requirements for goodness of fit: gamma ($p = 0.4333$), quantal-linear model ($p = 0.5784$), Weibull model ($p = 0.4312$), multistage ($p = 0.4055$), log-logistic ($p = 0.7766$), and log-probit ($p = 0.7368$). The log-logistic model was chosen to estimate the BMD_{10} and $BMDL_{10}$ because it had the lowest AIC and best visual fit at the control and two lowest doses, which encompassed the 10% response. The BMD_{10} and $BMDL_{10}$ for the log-logistic model were 10.57 and 5.6 mg/kg/day, respectively (see Appendix B).

Table 7. Incidence of forestomach lesions in female F344 rats

Administered dose (mg/kg/day)	Duration-adjusted dose (mg/kg/day)¹	Incidence of forestomach lesions
0	0	0/10
10	7	0/10
19	14	2/10
38	27	5/10
75	54	9/10
150	107	9/10

¹ Conversion to adjust for exposure duration (5 days to 7 days), e.g., 150 mg/kg/day \times 5/7 = 107 mg/kg/day.

5.1.3. RfD Derivation, Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)

Uncertainty factors (UFs) are applied to the BMD_{10} and $BMDL_{10}$ to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. Historically, UFs were applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF such as $10^{1/2}$, or 3 (U.S. EPA, 1994b), under conditions where toxicokinetics and mechanistic information are available and/or data are available on the nature and extent of human variability.

Chronic studies are preferred for RfD development. To account for the uncertainty in using a subchronic study for RfD derivation, the default UF of 10 is usually applied; however, for HCCPD, the ratio of subchronic to chronic NOAELs for the inhalation studies is used to determine the subchronic-to-chronic UF for oral exposure. This approach is justified by the fact that the most sensitive effect is a portal-of-entry effect for both routes of exposure. Respiratory effects and forestomach lesions are seen following inhalation and oral exposure, respectively. Thus, the differences in subchronic versus chronic dose-metrics are considered similar between the two routes of exposure. The subchronic inhalation study of NTP (1994) observed a NOAEL of 1.7 mg/m^3 for respiratory effects in rats while the chronic study observed a NOAEL of 2.23 mg/m^3 . Because comparing the subchronic NOAEL for inhalation exposure in rats to the chronic NOAEL yielded counterintuitive results, i.e., the subchronic NOAEL was less than the chronic NOAEL, the mouse results were examined. The subchronic mouse bioassay (NTP, 1994) yielded a NOAEL of 1.7 mg/m^3 whereas the NOAEL in the chronic assay was 0.56 mg/m^3 HCCPD. Thus, the subchronic:chronic ratio for NOAELs in mice is 3. It is more typical for the subchronic NOAEL to be larger than the chronic NOAEL, so 3, or $10^{1/2}$, was chosen as the subchronic-to-chronic UF for the RfD.

The toxicokinetics of HCCPD are not well understood, and it is not known if the toxicity is due to the parent compound or to metabolites. However, it is known that HCCPD does not bioaccumulate, and tissue concentrations and excretion of the compound depend somewhat on the exposure route. Rodent and rabbit studies show that oral HCCPD is absorbed rather poorly and excreted largely in the feces (about 70% of a single dose), but because there is no information on which to base a pharmacokinetic or pharmacodynamic comparison of animals to humans, the default UF of 10 is used for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibilities to HCCPD, so the default UF of 10 is used to protect sensitive human subpopulations.

The database for HCCPD includes studies of genotoxicity, developmental toxicity, systemic toxicity, and cancer. Although the three developmental studies (Murray et al., 1980; Chernoff and Kavlock, 1983; Goldenthal et al., 1978) were negative for structural defects and the histopathological observations for reproductive organs in the primary study (Abdo et al., 1984) were negative, functional information that would be provided by a reproductive toxicity or two-generation reproductive toxicity study is lacking. Absence of pathology does not necessarily

imply proper function. An additional UF of $10^{1/2}$ is added for these database deficiencies. Additional data that would increase confidence in the assessment include immunotoxicity, acute and subchronic neurotoxicity, and developmental neurotoxicity.

The total UF is 1,000 ($10^{1/2}$ for subchronic to chronic NOAEL, 10 for interspecies variability, 10 for intraspecies variability, and $10^{1/2}$ for database deficiency). The BMD_{10} and $BMDL_{10}$ are divided by 1,000 to derive the RfD.

$$BMD_{10} = 10.6 \div 1,000 = 0.011 \text{ mg/kg/day}$$
$$BMDL_{10} = 6 \div 1,000 = 0.006 \text{ mg/kg/day}$$

Thus, the RfD, as derived from the $BMDL_{10}$, is 0.006 mg/kg/day.

5.2. INHALATION REFERENCE CONCENTRATION

5.2.1. Choice of Principal Study and Critical Effect with Rationale and Justification

Only one chronic inhalation study for HCCPD was identified. NTP (1994) exposed rats and mice to 0, 0.11, 0.56, and 2.23 mg/m³ for 5 days/week for 2 years. Exposure to HCCPD did not affect survival in rats or in male mice. The survival of female mice in the 2.23 mg/m³ group was marginally lower than controls. Squamous metaplasia of the larynx was noted in female rats at 0.11 and 2.23 mg/m³ HCCPD, but it was not dose-related. No adverse effects were noted in male rats. Exposure-related effects in mice included suppurative inflammation of the nose in both sexes at 2.23 mg/m³.

Female mice exhibited suppurative inflammation of the ovaries that increased in a dose-dependent fashion. The effect was observed at 0.11 mg/m³ HCCPD, but began to be statistically significant at 0.56 mg/m³. The slightly lower survival rate for female mice in the 2.23 mg/m³ group was attributed to the ovarian inflammation. It was not considered to be the critical effect because it was a common occurrence thought to be due to pathogens thriving under inadequate sanitation procedures (Rao et al., 1987) and because several subchronic inhalation studies (NTP, 1994; Clark et al., 1982; Rand et al., 1982a) had identified the respiratory system as the major target of HCCPD toxicity.

The yellow-brown pigmentation of the respiratory epithelium was considered to be a marker of exposure rather than a toxic effect because it was not associated with any discernible lesion even after prolonged exposure. Although the designation of this pigmentation as nonadverse conflicts with ATSDR's treatment (ATSDR, 1999), it is consistent with the guidance in the RfC methodology (U.S. EPA, 1994b), which indicates that "enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects" should be ranked low in severity. Furthermore, the guidance states that "effects that may be considered marginal are designated as adverse only to the extent that they are consistent with other structural and functional data suggesting the same

toxicity,” indicating pigmentation does not qualify as an adverse effect in this situation. NTP (1994) suggested that lipid peroxidation may have produced the pigmentation.

Suppurative inflammation of the nose in mice was used as the critical endpoint for calculation of the RfC. The dose-response data for male and female mice from NTP (1994) and the duration adjustment to continuous exposure are shown in Table 8. The NOAEL for suppurative inflammation of the nose was 0.56 mg/m³ and the LOAEL was 2.23 mg/m³.

Table 8. Incidence of suppurative inflammation of the nose in mice

Exposure concentration (mg/m³)	Duration-adjusted exposure (mg/m³)¹	Nasal inflammation incidence
0	0	4/99
0.1	0.02	0/100
0.56	0.10	4/100
2.23	0.40	76/98

¹Conversion from intermittent exposure to continuous exposure:
 $0.56 \text{ mg/m}^3 \times 6/24 \text{ hrs} \times 5/7 \text{ days} = 0.10 \text{ mg/m}^3$.

Adjusting from intermittent to continuous exposure results in a duration-adjusted NOAEL of 0.1 mg/m³ and a LOAEL of 0.4 mg/m³.

5.2.2. Methods of Analysis—NOAEL/Benchmark Concentration Analysis

Benchmark concentration (BMC) analysis is preferred for dose-response analysis because it uses the entire dose-response curve to identify the point of departure, it does not depend upon dose spacing, and it is sensitive to the number of animals used in the study. The available data, however, did not meet the suggested criteria (U.S. EPA, 1995) of at least three dose levels with two doses eliciting a greater than minimum and less than maximum response. Thus, the duration-adjusted NOAEL of 0.10 mg/m³ is used to derive the RfC.

HCCPD is a Category 1 gas (U.S. EPA, 1994b) because its inhalation effects target the respiratory tract. The human equivalent concentration (HEC) for HCCPD is derived by multiplying the duration-adjusted NOAEL for rodents by an interspecies dosimetric adjustment factor for gas:respiratory effects in the region of critical effect. Because the critical effect is in the nose, the dosimetric adjustment factor was calculated for the extrathoracic (ET) region.

For HCCPD, the dosimetric adjustment factor is the regional gas dose ratio (RGDR) for HCCPD in the ET region. The RGDR was calculated as the ratio of mouse to human ventilation rate/ET surface area. The ventilation rate (V_E) was calculated for mice using the average body weight of males and females in the NOAEL exposure group (41.4 g). The ventilation rate for

mice was calculated as 0.049 L/minute using the allometric relationships contained on page 4-27 of U.S. EPA (1994b). The default human ventilation rate is 13.8 L/minute (U.S. EPA, 1994b). The default ET surface areas (SA_{ET}) for the mouse and for the human are shown in Table 4-4 of U.S. EPA (1994b) as 3.0 and 200 cm^2 , respectively. The RGDR was calculated as follows:

$$RGDR_{ET} = (V_E / SA_{ET})_{animal} / (V_E / SA_{ET})_{human} = (0.049/3.0) / (13.8/200) = 0.237$$

The duration-adjusted NOAEL was then multiplied by the $RGDR_{ET}$ to yield the $NOAEL_{HEC}$:

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR_{ET} = 0.1 \text{ mg/m}^3 \times 0.237 = 0.024 \text{ mg/m}^3$$

5.2.3. RfC Derivation Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)

Uncertainty factors (UFs) are applied to the $NOAEL_{HEC}$ to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. Historically, UFs were applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF such as $10^{1/2}$ (U.S. EPA, 1994b) under conditions where toxicokinetics and mechanistic information are available and/or data are available on the nature and extent of human variability.

For long-term rodent bioassays, the default UFs for interspecies extrapolation and within-species variability are each 10. Half of that factor, $10^{1/2}$, or 3, reflects the pharmacokinetic component of uncertainty and half represents the pharmacodynamic component of uncertainty. The calculation of an HEC adjustment to the NOAEL reduces the uncertainty associated with interspecies variation. Therefore, the use of $UF = 10^{1/2}$, instead of the default $UF = 10$, is justified for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibility; therefore, the default UF of 10 is used for within-species variation.

Although the available chronic and subchronic inhalation studies survey portal-of-entry and many systemic effects, the inhalation database is limited by the lack of information for developmental and reproductive toxicity. Thus, an additional uncertainty of $10^{1/2}$ for database deficiencies is used in the calculation of the RfC. Additional data that would increase confidence in the assessment include immunotoxicity, acute and subchronic neurotoxicity, and developmental neurotoxicity.

A total uncertainty factor of 100 ($10^{1/2}$ for interspecies variability, 10 for intraspecies variability, and $10^{1/2}$ for a limited database) is applied to the $NOAEL_{HEC}$ of 0.024 mg/m^3 , yielding an RfC of 0.0002 mg/m^3 .

5.3. CANCER ASSESSMENT

Human occupational studies and animal studies have failed to demonstrate an association between exposure to HCCPD and cancer. The NTP conducted a 2-year inhalation study with rats and mice and concluded that HCCPD exhibited no evidence of carcinogenic activity (NTP, 1994). HCCPD is not likely to be a human carcinogen because of the absence of increased deaths from cancer in limited human studies, no evidence of cancer in rodents, and lack of mutagenicity. Therefore, a quantitative dose-response assessment for carcinogenicity has not been conducted for HCCPD.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ASSESSMENTS

6.1. HAZARD IDENTIFICATION

HCCPD is a dense oily liquid, pale yellow to amber in color. It has a pungent, unpleasant odor. It is predominantly used as an intermediate in production of many dyes, resins, pharmaceuticals, flame retardants, insecticides, and polyester resins. HCCPD is also used to produce ketones, fluorocarbons, acids, esters, and shockproof plastics.

In animals, HCCPD is absorbed poorly after oral exposures, but is absorbed readily following inhalation exposures. Oral HCCPD is excreted mainly in the feces whereas inhaled HCCPD is excreted primarily in the urine. Metabolism is poorly characterized. The distribution of the compound and metabolites depends somewhat upon exposure route, but the kidneys, liver, and lungs are the major tissues of concentration regardless of route of exposure. HCCPD and metabolites are typically excreted within a few days of dosing and do not accumulate in tissues.

Although the data are limited, the repeated-exposure human toxicity data for HCCPD show no significant health effects. In animals, the compound adversely affects the histopathology of the tissues along the portal of entry. Inhalation exposure produces inflammation and hyperplasia in the nose, larynx, trachea, and lung of treated rodents exposed for 13 weeks at doses as low as 4.5 mg/m³ (NTP, 1994). A longer term study using lower doses found only suppurative inflammation of the nose at doses as low as 2.3 mg/m³. Gavage administration for 13 weeks induced mild to moderate forestomach lesions and toxic nephrosis in rats and mice (Abdo et al., 1984). The lowest dose producing these effects was 19 mg/kg. No significant developmental effects were observed via oral exposure in three studies using mice, rats, or rabbits at doses as high as 75 mg/kg during organogenesis (Goldenthal et al., 1978; Murray et al., 1980; Chernoff and Kavlock, 1983).

The potential carcinogenic effects of HCCPD have been studied in rodents (NTP, 1994). In a 2-year study that exposed rats and mice via inhalation, no treatment-related neoplastic lesions were observed. Generally, in vitro and in vivo mutagenicity tests have produced negative results. According to the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), HCCPD is most appropriately characterized as a Group E, Evidence of Noncarcinogenicity to Humans, carcinogen when exposure occurs by inhalation. This

characterization is based on inadequate data for cancer in humans and evidence of noncarcinogenicity in animals. In accordance with U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), HCCPD is not likely to be a human carcinogen via inhalation because of the absence of increased deaths from cancer in limited human studies, no evidence of noncarcinogenicity in rodents, and lack of mutagenicity. Because the existing chronic health effect data in both humans and animals do not include the inhalation route of exposure, the potential for carcinogenicity by the oral route is indeterminate.

6.2. DOSE RESPONSE

The RfD of 0.006 mg HCCPD/kg/day was derived from a 13-week subchronic bioassay (Abdo et al., 1984), in which rats and mice exhibited forestomach histopathology at the highest three doses tested. Forestomach lesions in female mice were identified as the critical effect. An overall uncertainty factor of 1,000 was applied to the BMDL₁₀ to account for the subchronic exposure, extrapolation from rat to human, intrahuman variability, and a limited database.

The overall confidence in the oral RfD is low; however, the confidence in the principal study is medium. Although the study was well conducted, an adequate number of doses were examined, and corroborative results in two species were obtained, no data on hematology, clinical chemistry, or urine analyses were collected. In addition, there are no supporting subchronic or chronic oral studies with which to compare the effects noted. Developmental studies are available for three species, but confidence in the database is low because of the lack of a chronic study and a two-generation reproductive study.

The developmental studies using oral administration of HCCPD during organogenesis reported no occurrence of adverse effects in mice, rats, or rabbits. Although these studies may suggest that HCCPD does not produce developmental effects, no multigenerational reproductive studies have been performed to examine effects on stages of development other than organogenesis.

The daily inhalation exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime (RfC) is 0.0002 mg/m³. This value was derived from a 2-year inhalation assay by NTP (1994). Dose-related suppurative inflammation of the nose was observed in mice. An overall uncertainty factor of 100 was used to account for the limited database, extrapolation from mouse to human, and intrahuman variability.

The overall confidence in the RfC assessment is medium. The confidence in the principal study is high because it was well designed and well conducted and followed standard guidelines for inhalation toxicity studies of chronic duration. The overall confidence in the database is medium. Although there are two subchronic studies that verify that the respiratory tract is the major target organ, the database lacks reproductive/developmental studies in rodents following inhalation exposure to HCCPD. Oral developmental studies in three species, however, indicate that HCCPD is not a developmental toxin at doses (i.e., 75 mg/kg) higher than those that cause

portal-of-entry irritation (i.e., 19 mg/kg). This suggests that the possible developmental effects of inhaled HCCPD may be less sensitive than respiratory tract effects.

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APPENDIX A. EXTERNAL PEER REVIEW— SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for HCCPD have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists outside EPA in accordance with EPA guidance on peer review (U.S. EPA, 1994). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

A. Appropriateness of the critical studies and critical effects for the RfD and RfC

All three reviewers agreed with the critical study (NTP, 1994) and the critical effect, suppurative inflammation of the nose, for RfC derivation. All reviewers agreed with the selection of Abdo et al. (1984) as the critical study for the RfD; however, one reviewer questioned the use of forestomach lesions as the critical effect. The reviewer suggested that the forestomach lesions were inappropriate because they resulted from acute irritation by the high concentration of the test compound in a corn oil vehicle. The reviewer suggested that the kidney toxicity seen in this study was a better critical effect because kidney toxicity is an expression of systemic injury from repeated and prolonged exposure and because it was consistent with the toxicokinetic data suggesting that the kidney is a major target organ.

Response to Comment: The forestomach lesions are believed to be a manifestation of chronic irritation, which is consistent with the observation of dermal irritation (Treon et al., 1955; Industrial Bio-test Laboratories, 1975a; HEW, 1978) and other portal-of-entry effects from HCCPD exposure (Clark et al., 1982; Rand et al., 1982a; NTP, 1994). Thus, Section 5.1.1 has been altered to characterize forestomach hyperplasia as a manifestation of chronic irritation. Another reviewer indicated that the observation of chronic irritation was supported by the fact that epithelial hyperplasia was found in association with inflammation.

B. Appropriateness of the uncertainty and modifying factors applied to the RfD and RfC

One reviewer agreed with all the uncertainty and modifying factors applied to the RfD and RfC. Another reviewer thought that a UF of 3 for database deficiency was unwarranted. The deficiency noted for the RfD was the absence of a two-generation reproductive study while those noted for the RfC were the absence of inhalation studies for both reproductive and developmental effects. The reviewer indicated that, for the RfD, there were three oral developmental toxicity studies that showed no reproductive effects and only maternal effects, so it was doubtful that a two-generation reproductive study would elicit any decrement of reproductive performance. For the RfC, this reviewer noted that the absorption data from inhalation studies indicated that blood levels were much less than those after developmental studies using oral administration, and that it was unlikely that an inhalation two-generation toxicity study could achieve blood and tissue levels capable of

producing reproductive studies when higher systemic doses did not do so in one-generation reproductive studies. The third reviewer suggested using 3 rather than 10 for the interspecies UF for the RfD because sensitivity to local chemical irritation is not likely to vary largely between species.

Response to Comment: The UF of 3 for database deficiency was originally added to the RfD to account for the lack of a two-generation reproductive study. Three developmental studies by the oral route (Murray et al., 1980; Chernoff and Kavlock, 1983; Goldenthal et al., 1978) and histopathological observations for reproductive organs in the primary study (Abdo et al., 1984) do provide information on pathology and histopathology of the reproductive organs, but the functional information that would be provided by a reproductive toxicity or two-generation reproductive toxicity study is lacking. Absence of pathology does not necessarily imply proper function. Other database deficiencies, including immunotoxicity, acute and subchronic neurotoxicity, and developmental neurotoxicity also provoke the application of the UF of 3. This rationale has been added to Section 5.1.3 to provide further support for the UF of 3 for database deficiencies.

It is acknowledged that the inhalation of 2.23 mg/m³ for 6 hours/day will deliver a much smaller dose of HCCPD/body weight than the oral developmental studies. Nevertheless, as noted above, no functional reproductive information is available even for the oral route. Other database deficiencies, including immunotoxicity, acute and subchronic neurotoxicity, and developmental neurotoxicity, also support the application of the UF of 3. This rationale has been added to Section 5.2.3 to provide further support for the UF of 3 for database deficiencies.

Even though it may be intuitively apparent that the sensitivity to local irritation would not vary greatly between species, there are no data that support this assumption. Thus, the interspecies UF for the RfD remains at 10.

C. Cancer Classification

All reviewers agreed with the group E carcinogen classification.

APPENDIX B. BENCHMARK DOSE CALCULATIONS FOR THE RfD

The RfD is based on forestomach lesions in the female rat, as reported in Abdo et al. (1984). The dose-response data and the conversion to continuous dosing are shown below in Table B-1.

Table B-1. Incidence of forestomach lesions in female F344 rats

Administered dose (mg/kg/day)	Continuous dose (mg/kg/day)	Incidence of forestomach lesions
0	0	0/10
10	7	0/10
19	14	2/10
38	27	5/10
75	54	9/10
150	107	9/10

¹ Conversion to adjust for exposure duration (5 days to 7 days),
e.g., 150 mg/kg/day x 5/7 = 107 mg/kg/day

NOAEL = 7 mg/kg/day

LOAEL = 14 mg/kg/day

The BMDL₁₀ (95% lowest confidence limit of the dose predicted to cause a 10% increase in the incidence of the effect) was estimated using U.S. EPA's Benchmark Dose Software (Version 1.2). The results of applying nine statistical models for dichotomous data from BMDS to the data for mild to moderate forestomach lesions are shown in Table B-2. Models with statistical goodness-of-fit *p*-value > 0.05 were ranked based on the values of the Akaike Information Criterion (AIC), a measure of the deviance of the model fit adjusted for the degrees of freedom, and evaluated for visual fit in the low-dose region, that approximates 10% response. The model with the lowest AIC and best visual fit is used to calculate the BMDL. The gamma, quantal-linear, Weibull, multistage, log-logistic, and log-probit models had adequate statistical goodness-of-fit. The log-logistic model results were used to derive the RfD because this model had the lowest AIC and the best visual fit at the control and two lowest doses, which encompassed the 10% response.

Table B-2. Benchmark dose results for forestomach lesions

Model	Chi-square goodness-of-fit <i>p</i>-value	AIC	Visual Rank	BMD₁₀ (mg/kg/day)	BMDL₁₀ (mg/kg/day)
Gamma	0.4333	44.6988	2	8.97	3.57
Logistic	0.0	NE	NE	24.8	24.3
Log-logistic	0.7766	42.8097	1	10.56	5.6
Multistage	0.4055	46.2565	4	5.41	3.13
Probit	0.0108	52.0664	NE	12.95	9.13
Log-probit	0.7368	42.9691	1	10.57	5.98
Quantal-linear	0.5784	44.5543	4	4.37	3.07
Quantal- quadratic	0.0000	NE	NE	14.44	11.82
Weibull	0.4312	45.3437	3	7.39	3.35

NE - Not evaluated because statistical goodness of fit *p*-value was < 0.05.

AIC - Akaike Information Criterion

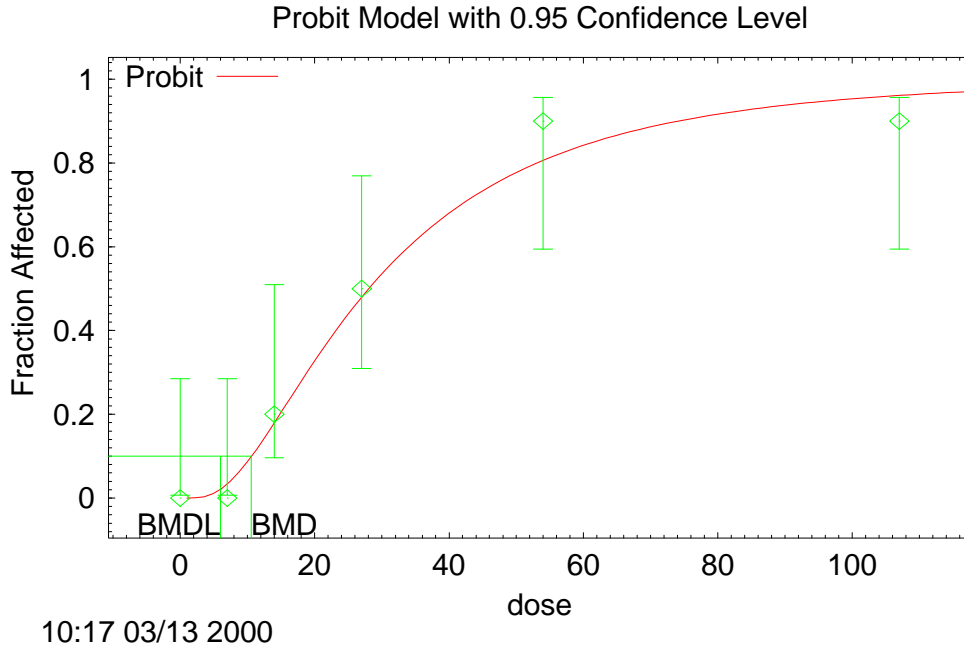
The BMD₁₀ of 10.6 mg/kg/day and the BMDL₁₀ of 6 mg/kg/day were divided by the UF of 1000 to derive the RfD.

$$\text{BMD}_{10} = 10.6 \div 1000 = 0.011 \text{ mg/kg/day}$$

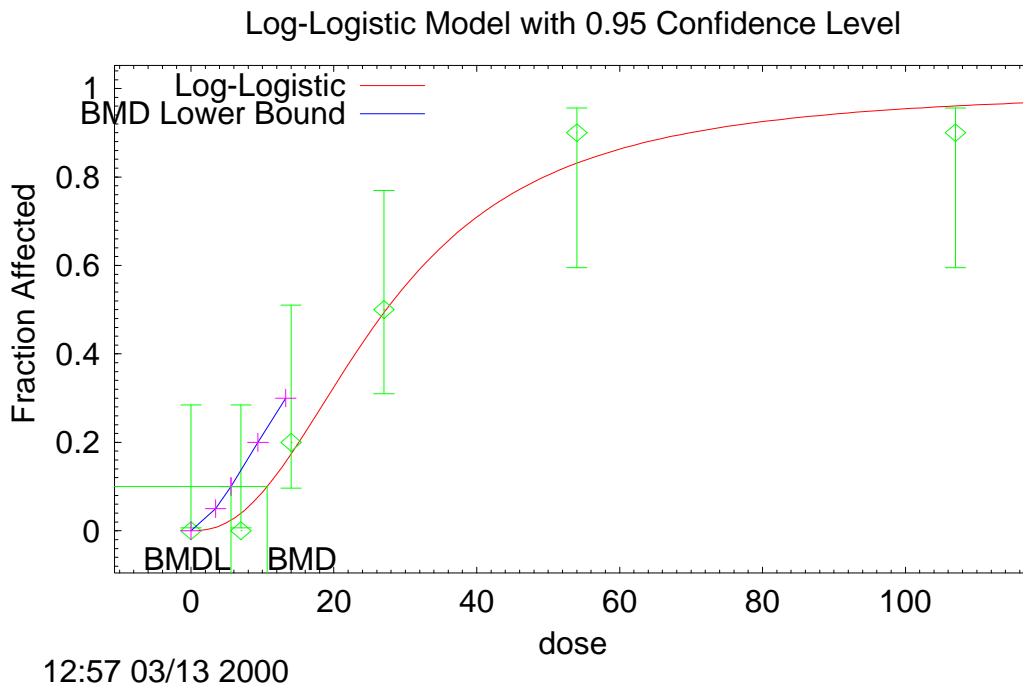
$$\text{BMDL}_{10} = 6 \div 1000 = 0.006 \text{ mg/kg/day}$$

Graphical results from the BMD models that were visually ranked follow.

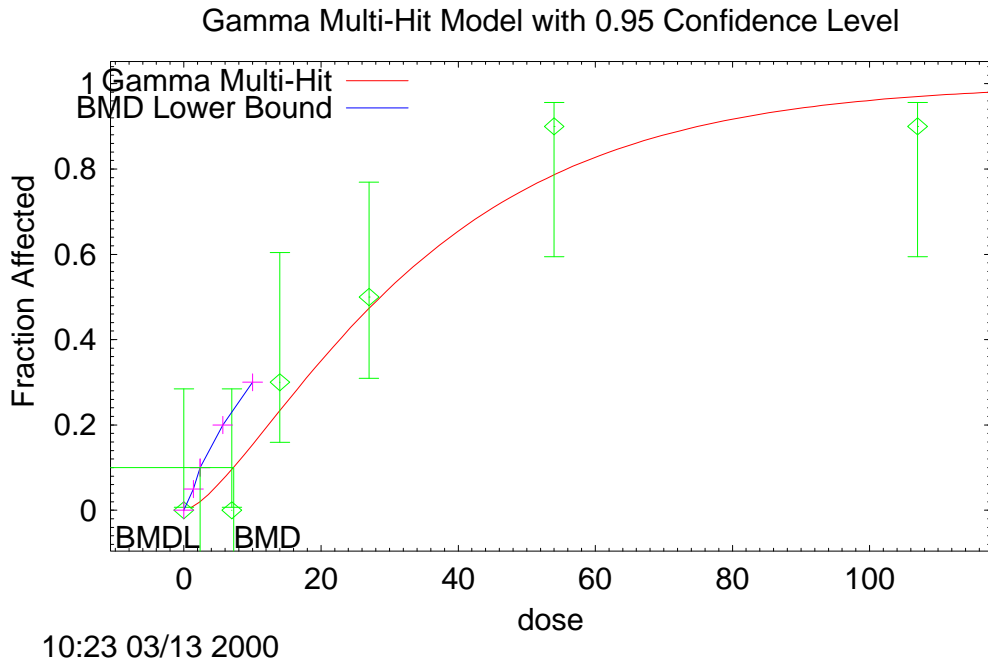
Log Probit-Visual rank=1



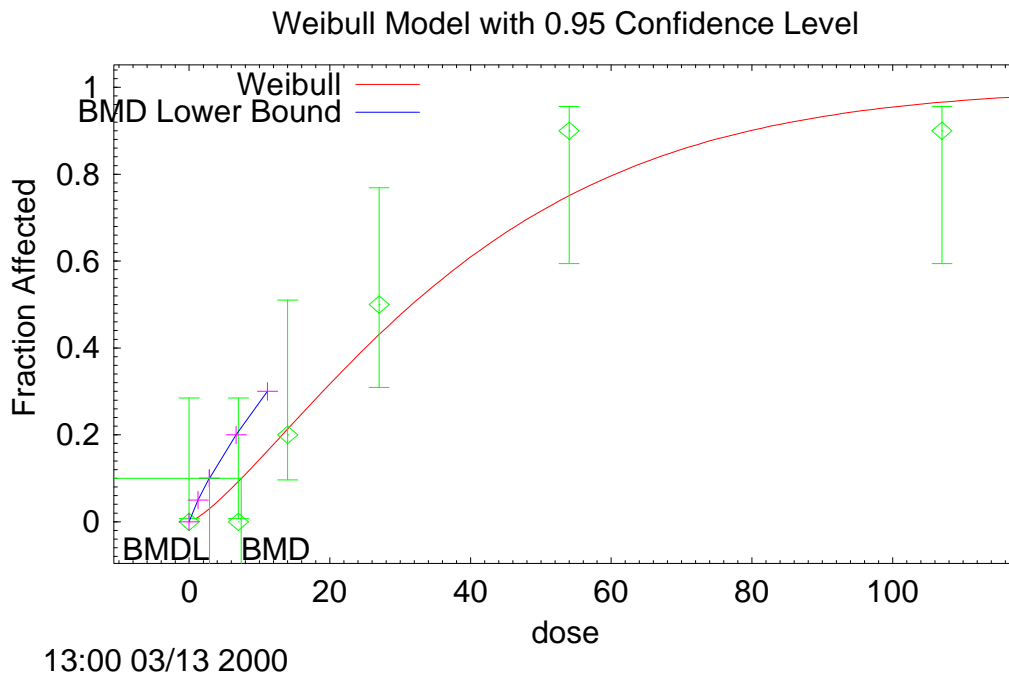
Log-logistic-Visual rank=1



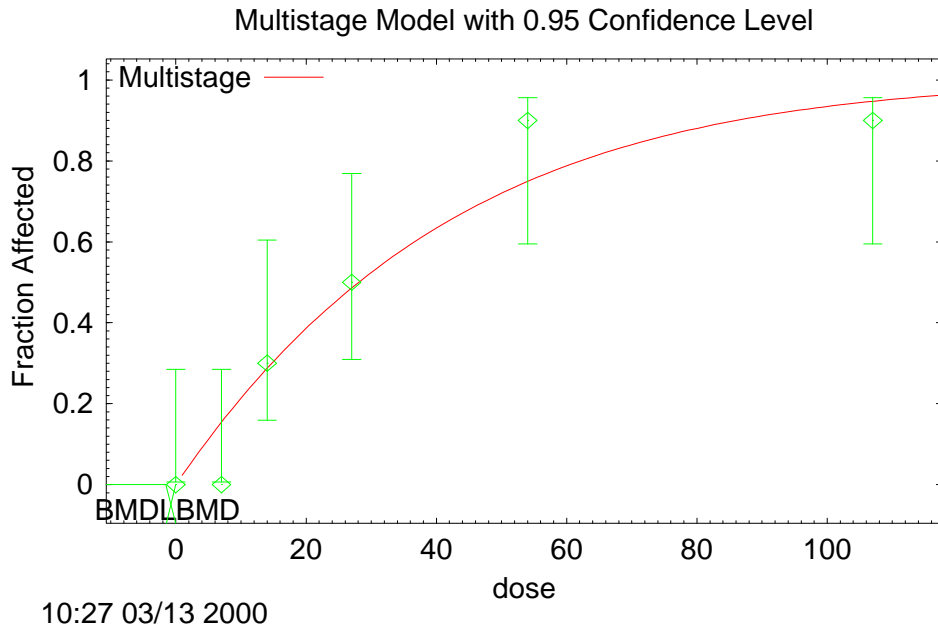
Gamma Multi-hit Visual rank=2



Weibull Visual rank=3



Multistage Visual rank=4



Quantal linear Visual rank=4

