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TOXICOLOGICAL REVIEW

OF

PENTACHLOROPHENOL

(CAS No. 87-86-5)

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

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NOTICE

U.S. Environmental Protection Agency
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TOXICOLOGICAL REVIEW OF PENTACHLOROPHENOL
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LIST OF ABBREVIATIONS AND ACRONYMS

AEL	acceptable exposure level
γ-GTP	γ-glutamyl transpeptidase
3MC	3-methylcholanthrene
8-OH-dG	8-hydroxy-2'-deoxyguanosine
AHH	arylhydrocarbon hydroxylase
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	alpha mouse liver
AP	apurinic
aPCP	analytical grade of PCP
AST	aspartate aminotransferase
AUC	area under the curve
BMD	benchmark dose
BMDL	95% lower bound of the BMD
BMR	benchmark response
BrdU	bromodeoxyuridine
BRI	biological reactive intermediate
BRL	Bionetics Research Laboratory, Inc.
BSA	bovine serum albumin
BUN	blood urea nitrogen
$BW^{3/4}$	body mass raised to the 3/4 power
CA	chromosomal aberration
CASRN	Chemical Abstracts Service Registry Number
CHO	Chinese hamster ovary
CI	confidence interval
CX	connexin
DEN	diethylnitrosamine
DETAPAC	diethylenetriamine pentaacetic acid
DMBA	dimethylbenzanthracene
DMSO	dimethylsulfoxide
DNP-Ficoll	2,4-dinitrophenyl-amincethylcarbamylmethyl-Ficoll
dUTP	deoxyuridine 5'-triphosphate
ED_{50}	median effective dose
EMCV	encephalomyocarditis virus
EMS	ethyl methanesulfonate
FSH	follicle stimulating hormone
GD	gestation day
GJIC	gap junction intercellular communication
GLP	Good Laboratory Practice
HAIR	hemolytic antibody isotope release
HCB	hexachlorobenzene
HED	human equivalent dose
HPRT	hypoxanthine phosphoribosyltransferase
HRP	horseradish peroxidase
HSDB	Hazardous Substances Data Bank

HxCDD	hexachlorodibenzo-p-dioxin
i.p.	interperitoneal(ly)
i.v.	intravenous
IARC	International Agency for Research on Cancer
ICD	International Classification of Disease
ID_{50}	median inhibitory dose
Ig	immunoglobulin
IL-8	interleukin-8
IQ	intelligence quotient
IRIS	Integrated Risk Information System
ISF	isosafrole
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LF	lipofuscin
LH	luteinizing hormone
LID	low iodine diet
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MCS	multiple chemical sensitivity
MLE	maximum likelihood estimate
MOA	mode of action
MSB	MSV-transformed tumor cell
MSV	Moloney sarcoma virus
MTD	maximum tolerated dose
ND	nondetectable
NHANES	National Health and Nutrition Examination Survey
NID	normal iodine diet
NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
NTP	National Toxicology Program
OCDD	octachlorodibenzo-p-dioxin
OPPTS	Office of Pollution, Prevention and Toxic Substances
OR	odds ratio
OuaR	ouabain resistance
PB	phenobarbital
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
PCP	pentachlorophenol
PFC	plaque-forming cell
POD	point of departure
RAL	relative adduct levels
RBC	red blood cell
RED	reregistration eligibility decision
RfC	reference concentration
RfD	reference dose
ROS	reactive oxygen species
RR	relative risk
SCE	sister chromatid exchange

SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SRBC	sheep red blood cell
SSB	single strand break
T_3	triiodothyronine
T_4	thyroxine
TCDD	tetrachlorodibenzo-p-dioxin
TCHQ	tetrachlorohydroquinone
TCoBQ	tetrachloro-o-benzoquinone
TCoHQ	tetrachloro-o-hydroquinone
TCoSQ	tetrachloro-1,2-benzosemiquinone
TCP	tetrachlorophenol
TCpBQ	tetrachloro p-benzoquinone
TCpCAT	tetrachlorocatechol
TCpHQ	tetrachloro-p-hydroquinone
TCpSQ	tetrachloro-1,4-benzosemiquinone
TGr	6-thioguanine resistance
TPA	tetradecanoylphorbol acetate
tPCP	technical grade of PCP
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_D	database deficiency uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
WBC	white blood cell

FOREWORD

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

The intent of Section 6, Major Conclusions in the Characterization of Hazard and Dose Response, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey 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 IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A.

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of pentachlorophenol (PCP). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as 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 (expressed in units of mg/m^3) 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 (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. 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 may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a plausible inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for PCP has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim*

Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through August 2009.

2. CHEMICAL AND PHYSICAL INFORMATION

PCP (CASRN 87-86-5) is a chlorinated aromatic compound that appears in a solid crystalline state and ranges in color from colorless to white, tan, or brown. The chemical, also referred to as penta, pentachlorofenol, 2,3,4,5,6-PCP, and chlorophen, has a phenolic odor that is pungent when heated. PCP is nonflammable and noncorrosive, and, although solubility is limited in water, it is readily soluble in alcohol (Budavari et al., 1996; NTP, 1989). The physical/chemical properties of PCP are summarized below (NLM, 1999a, b; Budavari et al., 1996; Allan, 1994; Royal Society of Chemistry, 1991).

Chemical formula	C_6HOCl_5
Molecular weight	266.34
Density	1.978 g/mL (at 22°C/4°C)
Melting point	190–191°C
Boiling point	~309–310°C
Water solubility	80 mg/L (at 20°C), 14 mg/L (at 26.7°C)
Log K_{ow}	5.01
Log K_{oc}	4.5
Vapor pressure	0.00011 (at 20°C)
Vapor density	9.20 (air = 1)
Henry's law constant	2.45×10^{-8} (atm × m^3)/mole
Conversion factors	1 ppm = 10.9 mg/m^3; 1 mg/m^3 = 0.09 ppm; 1 ppm = 0.01088 mg/L; 1 mg/L = 99.1 ppm (at 25°C)

PCP was first registered in the United States in 1936 as a wood preservative to prevent decay from fungal organisms and insect damage (Ahlborg and Thunberg, 1980). It was widely used as a biocide and could also be found in ropes, paints, adhesives, canvas, insulation, and brick walls (Proudfoot, 2003; ATSDR, 2001). After use by the general public was restricted in 1984, PCP application was limited to industrial areas (e.g., utility poles, cross arms, railroad cross ties, wooden pilings, fence posts, and lumber/timbers for construction). Currently, products containing PCP remain registered for wood preservation; utility poles and cross arms represent approximately 92% of all uses for PCP-treated lumber.

PCP is produced via two pathways, either "by stepwise chlorination of phenols in the presence of catalysts (anhydrous aluminum chloride or ferric chloride) or alkaline hydrolysis of [hexachlorobenzene] HCB" (Proudfoot, 2003). In addition to industrial production of PCP, the degradation or metabolism of HCB (Rizzardini and Smith, 1982), pentachlorobenzene (Kohli et al., 1976), or pentachloronitrobenzene (Renner and Hopfer, 1990) also yields PCP. Impurities found in PCP are created during the production of the chemical. The technical grade of PCP

(tPCP), frequently found under the trade names Dowicide 7, Dowicide EC-7 (EC-7), Dow PCP DP-2 Antimicrobial (DP-2), Duratox, Fungol, Penta-Kil, and Permacide, is composed of approximately 90% PCP and 10% contaminants. The impurities consist of several chlorophenol congeners, chlorinated dibenzo-p-dioxins, and chlorinated dibenzofurans. Of the chlorinated dibenzo-p-dioxin and dibenzofuran contaminants, the higher chlorinated congeners are predominantly found as impurities within tPCP. In addition to the chlorinated dibenzo-p-dioxin and dibenzofuran contaminants, HCB and chlorophenoxy constituents may also be present in tPCP. Use of the analytical grade of PCP (aPCP) first requires a purification process to remove the contaminants that were created during the manufacturing of PCP. The physicochemical properties of these contaminants are listed in Appendix B in Tables B-1 and B-2.

Grades described as analytical or pure are generally ≥98% PCP and the levels of dioxins and furans are low to nondetectable. Purities of technical- and commercial-grade PCP formulations are reported to be somewhat less than the analytical formulations, ranging from 85 to 91%. Hughes et al. (1985) reported that tPCP contains 85–90% PCP, 10–15% trichlorophenol, and tetrachlorophenol (TCP), and <1% chlorinated dibenzo-p-dioxin, chlorinated dibenzofurans, and chlorinated diphenyl ethers. The compositions of different grades of PCP as reported by the National Toxicology Program (NTP) (and similar to values reported in the general literature) are listed in Table 2-1.

Table 2-1. Impurities and contaminants in different grades of PCP

Contaminant/impurity[a]	Pure/analytical	Technical grade	DP-2	Dowicide EC-7
PCP	98.6%	90.4%	91.6%	91%
Chlorophenols				
Dichlorophenol	–	–	0.13%	–
Trichlorophenol	<0.01%	0.01%	0.044%	0.007%
TCP	1.4%	3.8%	7.0%	9.4%
HCB	10 ppm	50 ppm	15 ppm	65 ppm
Dioxins				
Tetrachlorodibenzodioxin	<0.08 ppm	–	–	<0.04 ppm
Pentachlorodibenzodioxin	–	–	–	–
Hexachlorodibenzodioxin	<1 ppm	10.1 ppm	0.59 ppm	0.19 ppm
Heptachlorodibenzodioxin	–	296 ppm	28 ppm	0.53 ppm
Octachlorodibenzodioxin	<1 ppm	1,386 ppm	173 ppm	0.69 ppm
Ethers				
Pentachlorodibenzofuran	–	1.4 ppm	–	–
Hexachlorodibenzofuran	–	9.9 ppm	12.95 ppm	0.13 ppm
Heptachlorodibenzofuran	–	88 ppm	172 ppm	0.15 ppm

Table 2-1. Impurities and contaminants in different grades of PCP

Contaminant/impurity[a]	Pure/analytical	Technical grade	DP-2	Dowicide EC-7
Octachlorodibenzofuran	–	43 ppm	320 ppm	–
Hexachlorohydroxydibenzofuran	0.11%	0.16%	0.07%	–
Heptachlorohydroxydibenzofuran	0.22%	0.47%	0.31%	–
Chlorohydroxydiphenyl ethers	0.31%	5.58%	3.67%	–

[a]The DP-2 and EC-7 commercial formulations are no longer manufactured and are listed for informational purposes only.

Source: NTP (1989).

3. TOXICOKINETICS

The toxicokinetics of PCP have been studied in both humans and animals. These studies show that PCP is rapidly and efficiently absorbed from the gastrointestinal and respiratory tracts (Reigner et al., 1992a, b, c). Once absorbed, PCP exhibits a small volume of distribution. Metabolism occurs primarily in the liver, to a limited extent, via oxidative dechlorination and conjugation. Tetrachlorohydroquinone (TCHQ) and the conjugation product, PCP-glucuronide, have been confirmed as the two major degradation products. PCP is predominantly excreted unchanged and found in the urine in the form of the parent compound. The low degree of metabolism is frequently attributed to extensive plasma protein binding.

3.1. PCP LEVELS IN GENERAL AND OCCUPATIONALLY EXPOSED POPULATIONS

Several reports have provided data on levels of PCP in blood or urine samples in humans (general population samples or groups with known exposures to PCP) indicating that PCP is absorbed in humans. The correlation between blood and urinary values is relatively high when the urinary data are corrected for creatinine clearance [0.92 in Cline et al. (1989) and 0.76 in Jones et al. (1986)]. Studies from Hawaii (Klemmer, 1972; Bevenue et al., 1967) and the United Kingdom (Jones et al., 1986) have demonstrated blood (plasma or serum) and urine values of PCP in workers with high PCP exposures (e.g., pesticide operators, wood treaters, and other wood workers) that are approximately an order of magnitude higher than in nonexposed groups within the same study.

People who lived or worked in buildings in which PCP-treated wood was used have been found to have mean serum levels up to 10 times higher than groups that were not exposed (Gerhard et al., 1999; Peper et al., 1999; Cline et al., 1989). Similar patterns were seen in the urinary data. Sex differences were not noted for the PCP serum levels in log home residents, but age differences were observed. Children ages 2–15 had serum PCP levels 1.7–2.0 times higher than those of their parents. Cline et al. (1989) attributed the higher PCP levels in children to differences in the ventilation rate to body weight ratio, although Treble and Thompson (1996) reported no age-related differences in urinary PCP concentrations in 69 participants ages 6–87 years (mean 54.6 years) living in rural and urban regions of Saskatchewan, Canada. See tables in Appendix C for further details on occupationally exposed humans.

Renner and Mücke (1986), in reviewing the metabolism of PCP, noted that establishing a direct relationship between PCP exposure levels and PCP in body fluids may be difficult because PCP is a metabolite of other environmental contaminants (e.g., HCB, pentachlorobenzene, pentachloronitrobenzene) and is itself metabolized.

Casarett et al. (1969) reported mean 10-day urine concentrations of 5.6 and 3.2 ppm in two groups of workers handling PCP under different conditions. The mean decrease in urine

concentration in workers following different periods of absence from their jobs was 39% within the first 24 hours and 60–82% over the next 17 days. Continued excretion of PCP was noted after 18 days of absence from the job. A semilog plot shows a linear relationship between plasma and urine concentrations at plasma concentrations of 0.1 ppm and a plateau for plasma concentrations >10 ppm.

In another experiment by Casarett et al. (1969), air concentrations, blood levels, and urinary excretion of PCP were measured 2 days before a 45-minute exposure and 5 days after exposure to PCP. Mean air concentrations of 230 and 432 ng/L (calculated doses were 90.6 and 146.9 µg, respectively) were associated with 88 and 76% excretion of PCP in the urine, respectively. Excretion was slow during the first 24 hours ($t_{1/2}$ = 40–50 hours) and more rapid after the first day ($t_{1/2}$ = 10 hours). In one subject, urine concentrations returned to baseline after 48 hours, but remained elevated in the other subject.

Begley et al. (1977) reported on blood and urine PCP levels in 18 PCP-exposed workers before, during, and after a 20-day absence from their jobs. Except for a brief rise on postexposure day 6, blood PCP levels during a 20-day absence showed a steady decline to 50% of the level measured on the last day of work (i.e., exposure). There was a 6-day lag in the decrease in urine level; after day 20, urine levels had decreased about 50%. Begley et al. (1977) also noted that the high PCP levels were accompanied by impaired renal function measured by creatinine and phosphorus clearance and phosphorus reabsorption.

Ahlborg et al. (1974) detected PCP, as well as the metabolites TCHQ and tetrachloropyrocatechol, in the urine of workers occupationally exposed to PCP. They did not quantify the levels of metabolites in urine.

3.2. ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

3.2.1. Oral Studies

3.2.1.1. *Absorption*

Braun et al. (1979) orally dosed four male human subjects with 0.1 mg/kg unlabeled PCP (ingested in 25 mL of water). The absorption half-life for the volunteers was 1.3 hours, with a maximum plasma concentration (C_{max}) of 0.245 µg/mL and a time to peak plasma concentration (T_{max}) of 4 hours. In another study, Braun et al. (1977) reported that the absorption rate constants for PCP administered in corn oil to Sprague-Dawley rats were 1.95 and 1.52 $hour^{-1}$ for males and females, respectively. The plasma T_{max} was 4–6 hours.

Larsen et al. (1975) observed that PCP levels (measured as percentage of administered dose of [^{14}C]PCP [99.54% radiochemical purity] and/or its metabolites per gram of tissue) peaked in maternal blood serum 8 hours after dosing 14 Charles River CD (Sprague-Dawley derived) rat dams with 60 mg/kg on gestation day (GD) 15 (administered in a solution of olive oil; 100 mg/6 mL). The serum levels, peaking at approximately 1.13% [^{14}C]PCP per gram of blood serum, steadily dropped during the remaining part of the 32-hour monitoring period for a

final measurement of 0.45% [^{14}C]PCP per gram of blood serum. [^{14}C]PCP in the placenta peaked at 0.28% of administered dose 12 hours after dosing. The level reaching the fetus peaked at 0.08% of the administered dose of [^{14}C]PCP and remained extremely low throughout the monitoring period. The levels of [^{14}C]PCP per gram of tissue measured in the placenta and fetus were much lower than those levels found in the maternal blood serum.

Reigner et al. (1991) studied toxicokinetic parameters in 10 male Sprague-Dawley rats administrated 2.5 mg/kg of aPCP (99% purity) via intravenous (i.v.) or gavage (five animals/route) routes. Absorption was rapid and complete, with 91% bioavailability after oral administration. Plasma levels peaked at 7.3 μg/mL after 1.5–2 hours and declined with a half-life of 7.5 hours. Reigner et al. (1992c) examined the pharmacokinetics of orally administered PCP (15 mg/kg) in male B6C3F$_1$ mice. The data were consistent with an open one-compartment model. Absorption followed first-order kinetics. Peak plasma concentration (28 μg/mL) was achieved at 1.5 hours. Absorption was complete; bioavailability was measured as 106%.

Yuan et al. (1994) studied the toxicokinetics of PCP (>99% purity) administered to F344 male rats by gavage (n = 18) at doses of 9.5 or 38 mg/kg, or dosed feed (n = 42) containing 302 or 1,010 ppm PCP (21 or 64 mg/kg-day, respectively) for 1 week. In addition, groups of 18 male and 18 female rats were administered PCP at a dose of 5 mg/kg by i.v. injection. Following gavage administration, the absorption half-life of 1.3 hours and plasma concentrations that peaked in approximately 2–4 hours indicated very rapid absorption from the gut. For the dosed feed study, absorption was also rapid and followed first-order kinetics. Plasma concentrations showed repeated cycles of peaks and troughs, coinciding with feeding cycles (i.e., highest concentrations at night and lowest during the day); however, plasma concentration did not reach pretreatment levels during the day. Absorption from the gut was estimated as 52 and 30% for administered doses of 21 (302 ppm) and 64 mg/kg-day (1,010 ppm), respectively. The bioavailability was much lower than the values obtained from the gavage study. The investigators noted that the lower bioavailability for the dosed feed study suggests that PCP interacts with components in feed. The data from the i.v. study were fitted to a two-compartment model. The investigators stated that absorption and elimination half-lives were not affected by the change from gavage to dosed feed administration.

Braun and Sauerhoff (1976) orally administered a single 10 mg/kg dose of [^{14}C]PCP to Rhesus monkeys in 10 mL of corn oil solution. The absorption kinetics of [^{14}C]PCP were first order with the absorption half-life ranging from 1.8 to 3.7 hours. Deichmann et al. (1942) reported that absorption was immediate and rapid in rabbits given a single 18 mg/kg oral dose of PCP (in feed), and peak blood levels were achieved 7 hours after dosing rabbits with 37 mg/kg PCP (in feed). Deichmann et al. (1942) administered 90 successive (except Sundays) oral doses of 0.1% PCP sodium salt (equivalent to 3 mg/kg) to 23 rabbits (sex not reported) in feed. Average peak blood concentrations of 0.6 mg PCP per 100 mL blood were measured within 4 days and did not change much for the remaining duration of the study. The investigators noted

that the blood concentrations of PCP were similar to those attained after 100 daily skin applications of 100 mg each (0.45 mg PCP per 100 mL of blood).

3.2.1.2. *Distribution*

Binding of PCP to specific components of liver cells or differential distribution of PCP to different cellular organelles may affect its metabolic fate. Arrhenius et al. (1977a) administered a 40 mg/kg dose of aPCP by gavage to rats; the animals were sacrificed 16 hours later. The relative concentration of PCP in microsomes was 6 times greater than in mitochondria. PCP acts as an inhibitor of mitochondrial oxidative phosphorylation (Weinbach, 1954) and has been shown to inhibit the transport of electrons between a flavin and cytochrome P450, thereby interrupting the detoxification enzyme system (Arrhenius et al., 1977a, b). Arrhenius et al. (1977a) suggested that inhibition of microsomal detoxification and inhibition of mitochondrial oxidative phosphorylation might be equally important.

Binding to plasma proteins plays a significant role in the distribution of PCP that likely affects the amount available for metabolism and clearance. Uhl et al. (1986) found that >96% of PCP was bound to plasma proteins in blood samples of three human males receiving an oral dose of 0.016 mg/kg PCP (dissolved in 40% ethanol). Gomez-Catalan et al. (1991) found 97 ± 2% of the administered dose of PCP (10–20 mg/kg in water and corn oil via gavage) bound to plasma proteins in rats. Braun et al. (1977) examined tissues of rats orally administered PCP (in corn oil) and showed the greatest accumulation of PCP in the liver and kidneys, with minimal levels in the brain and fat. The study demonstrated that plasma protein binding accounted for approximately 99% of the PCP. The authors noted that tissue/plasma ratios and renal clearance rates following oral administration of PCP were much lower than would be predicted based on the octanol/water coefficient and the glomerular filtration rate and suggested that the plasma protein binding resulted in low renal clearance and tissue accumulation.

3.2.1.3. *Metabolism*

Studies in animals and humans indicate that PCP is metabolized primarily in the liver. However, PCP is not extensively metabolized; a large portion of the administered dose is excreted unchanged in the urine. The major metabolic pathways are oxidative dechlorination to form tetrachloro-p-hydroquinone (TCpHQ, also reported as TCHQ) and conjugation with glucuronide. Extensive plasma protein binding occurs that may account, at least in part, for the low degree of metabolism.

Braun et al. (1979) measured 86% of the administered dose of PCP (0.1 mg/kg; ingested in 25 mL of water) in the urine and 4% in feces of four human males 8 days after ingestion of PCP. The study reported that human male subjects excreted 74 and 2% of the administered dose in urine and feces, respectively, as unmetabolized PCP. PCP, as the conjugated glucuronide, was measured as 12 and 2% of the administered dose in urine and feces, respectively. TCpHQ was not identified.

Ahlborg et al. (1974) detected PCP, as well as the metabolites TCHQ and tetrachloropyrocatechol, in the urine of workers occupationally exposed to PCP. They did not quantify the levels of metabolites in urine. Uhl et al. (1986) found PCP-glucuronide conjugate accounted for about 28% of the PCP in the urine of human males on day 1 and about 60% from days 15 to 38 after dosing with 0.31 mg/kg PCP (dissolved in 40% ethanol). The percentage of PCP-glucuronide conjugate measured in this study is similar to reported levels in urine of nonoccupationally exposed people. Although previous studies found urinary metabolites TCHQ and TCP in humans, and TCHQ in animals (Kalman, 1984; Edgerton et al., 1979; Ahlborg et al., 1974), the authors noted that the data showed no traces of these metabolites of PCP.

Mehmood et al. (1996) studied the metabolism of PCP (purity not reported) in microsomal fractions and whole cells of *Saccharomyces cerevisiae* expressing human CYP3A4. PCP was transformed to TCpHQ, although, in contrast to expected results, further hydroxylations were not observed. In transformed animals in which CYP3A4 was lacking, metabolism of PCP was not detected. In humans, this enzyme has low activity in the first month of life, but approaches adult levels by 6–12 months of age. Adult activity may be exceeded between 1 and 4 years of age, although activity usually declines to adult levels at the end of puberty. Functional activity of CYP3A7 in the fetus is approximately 30–75% of adult levels (Leeder and Kearns, 1997). aPCP (>99%) was identified as an inducer of CYP3A7 in studies in cultured rat hepatocytes, quail hepatocytes, and human hepatoma (Hep G2) cells (Dubois et al., 1996).

Juhl et al. (1985) studied the metabolism of PCP in human S9 liver fractions from biopsy patients and compared the results with those obtained from S9 liver preparations from noninduced and Aroclor 1254-induced male Wistar rats. Human S9 fractions converted PCP to TCpHQ. Maximum conversion occurred after incubation for 3 hours, after which the level of TCpHQ steadily declined to nondetectable levels at 24 hours. The authors attributed the decline to the oxidation capacity of the liver preparation or the further oxidation of TCpHQ to semiquinone radicals. The patterns of conversion of PCP to TCpHQ in human and rat liver S9 preparations showed very little difference. Juhl et al. (1985) and the more recent study by Mehmood et al. (1996) report the formation of the TCHQ metabolite of PCP in human liver tissue and are supportive of the earlier findings of Ahlborg et al. (1974), Edgerton et al. (1979), and Kalman (1984).

Braun et al. (1977) administered 10 or 100 mg/kg [^{14}C]PCP (in corn oil) to rats. After administration of a 10 mg/kg dose, approximately 80% of the dose was excreted in urine and about 19% was excreted in feces of both male and female rats. After administration of 100 mg/kg, males excreted 72% of the administered dose in urine and 24% in feces (which is similar to the excretion measured in male and female rats administered 10 mg/kg), whereas 100 mg/kg females excreted 54% in urine and 43% in feces. The reason for the difference in excretion in the females administered the higher dose of PCP is unknown; however, the decrease

in the amount of PCP excreted in urine is likely reflected in the increase in amount of PCP excreted in the feces, relative to that observed in the males at 100 mg/kg and male and female rats at 10 mg/kg. Expired air accounted for a small amount of the administered dose. Unmetabolized PCP accounted for 48% of the administered dose in urine; TCHQ and PCP-glucuronide conjugate accounted for 10 and 6%, respectively.

PCP metabolites were measured in urine and feces from male Wistar rats administered 8 mg/kg-day PCP by gavage for 19 days (Engst et al., 1976). Under these conditions, most of the PCP in urine was unmetabolized; small amounts of 2,3,4,5-TCP, 2,3,4,6-TCP and/or 2,3,5,6-TCP, and 2,3,4-trichlorophenol were found. No metabolites and only a small amount of unmetabolized PCP were identified in feces.

Van Ommen et al. (1986a) studied the in vitro metabolism of PCP (100 μM) utilizing rat liver microsomal preparations from untreated male and female Wistar rats and from rats treated with HCB, phenobarbital (PB), 3-methylcholanthrene (3MC), or isosafrole (ISF). Rat liver microsomes converted PCP only to TCpHQ and tetrachloro-1,2-hydroquinone (TCoHQ) via cytochrome P450 enzymes. The conversion rate (pmol total soluble metabolite formed per mg protein per minute) increased sevenfold in rat microsomes induced with ISF and three- to fourfold in HCB-induced rats. PB and 3MC increased the conversion rate two- to threefold over the controls. The ratios of TCpHQ/TCoHQ production were 4.9:1 for male rats and 1.6:1 for female rats receiving no inducer. The ratio decreased in rats treated with the enzyme inducers in the following order: HCB >PB >3MC ≈ ISF. The sex difference observed in untreated rats was not observed in rats treated with the inducers, although there was no change in the conversion rate in female rats (as opposed to male rats) treated with PB.

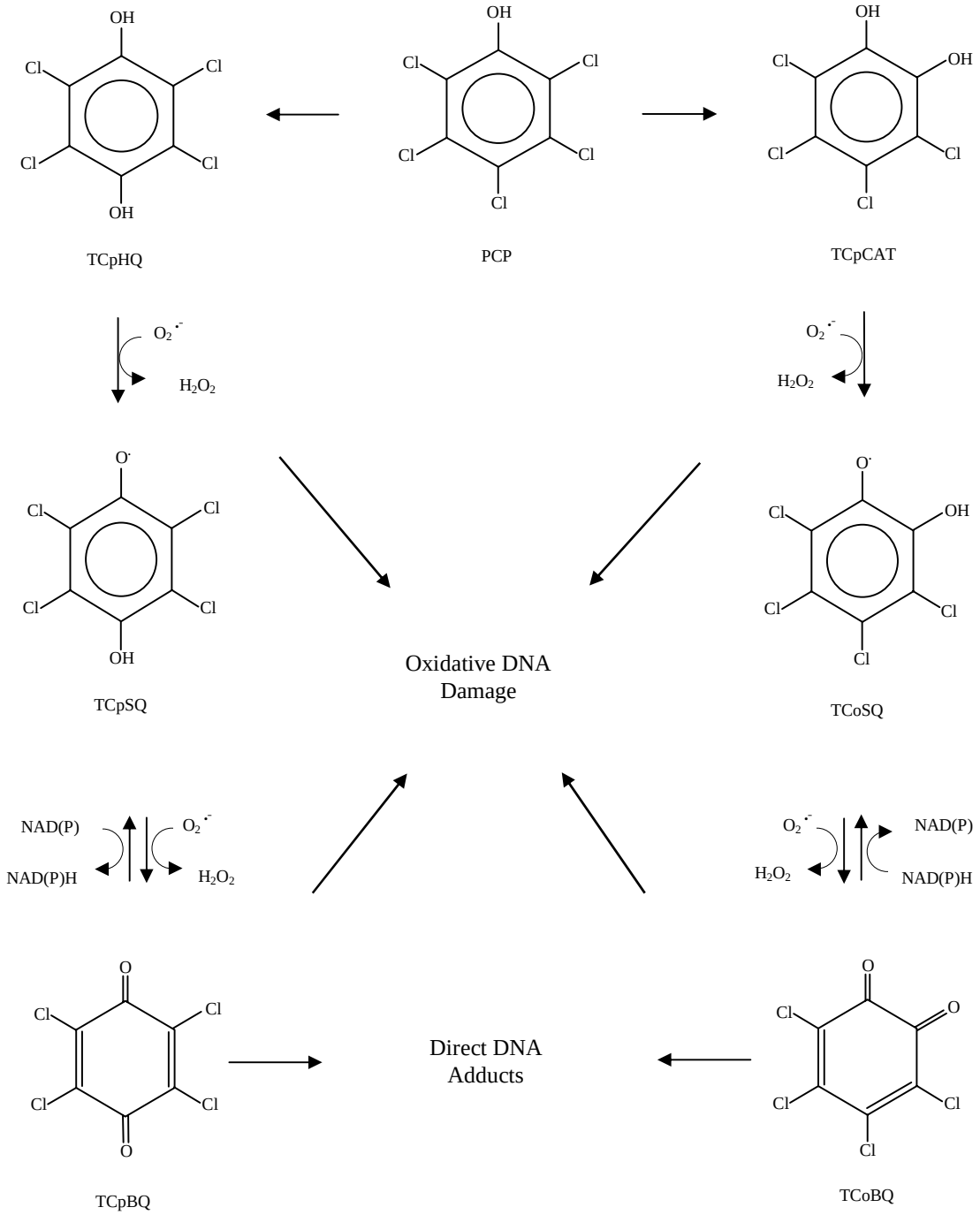
Van Ommen et al. (1986b) found that PCP binds to microsomal proteins. Protein binding was dependent on metabolism, and the amount bound did not vary considerably with the microsomal preparations (63–75 pmol/mg protein-minute) except for that obtained from PB-induced female rats (104 pmol/mg protein-minute). Van Ommen et al. (1986b) indicated that the "benzoquinone or the semiquinone form" of TCpHQ and TCoHQ "is responsible for the covalent binding properties." Protein binding was inhibited by glutathione through conjugation with benzoquinone. When the covalent binding was inhibited through reduction of benzoquinones and semiquinones to the hydroquinone form by ascorbic acid, the formation of TCpHQ and TCoHQ increased. DNA binding also occurred, but to a lesser degree than protein binding. Covalent binding to DNA was 12 ± 3 pmol/mg DNA-minute, while the average microsomal protein binding was 63 pmol/mg protein-minute. The K_m value for covalent binding to protein and conversion to hydroquinone was 13 μM, and the authors suggested that these activities resulted from the same reaction (Van Ommen et al., 1986a).

Tsai et al. (2001) attempted to analyze two proposed pathways of PCP (purity not reported) metabolism. Additionally, the authors were interested in illustrating any differences in metabolism between rats and mice that may explain the varied tumor patterns observed in the

two species of rodents (NTP, 1999, 1989). One potential metabolism pathway involves cytochrome P450-mediated dechlorination of PCP to TCHQ and TCpCAT which are oxidized to the respective benzoquinones and semiquinones in both Sprague-Dawley rats and B6C3F_1 mice. Alternatively, PCP is oxidized via peroxidase to tetrachloro-p-benzoquinone (TCpBQ) by a direct P450/peroxidase-mediated oxidative pathway. The formation of tetrachloro-o-benzoquinone (TCoBQ) via the latter pathway has not been verified.

Tsai et al. (2001) found that liver cytosol and cumene hydroperoxide in either the presence or absence of microsomes activated PCP and resulted in a greater production of PCP-derived adducts (quinones or semiquinones) than when PCP was activated with microsomes and NADPH. The investigators demonstrated that induction of microsomes, via 3MC or PB, led to PCP metabolism resulting in the formation of TCpBQ in both rats and mice. Increased metabolism to the adduct-forming benzoquinones following induction by 3MC and PB was observed in both rats and mice, although the mice exhibited an increase in BQ adduct formation that was significantly greater than that in rats. Other adducts measured, such as TCpBQ, did not exhibit an induction greater than the controls. Results of this study as well as others (Mehmood et al., 1996; Van Ommen et al., 1986a) indicate that various isozymes of P450 are responsible for metabolism of PCP. The authors "speculate that the increased 3MC-related induction of specific P450 isozymes in mice (eightfold increase versus control) compared with rats (2.4-fold increase versus control), may have played a role in the formation of liver tumors in mice (but not rats) dosed with PCP."

Lin et al. (2002) proposed a metabolism pathway for PCP (Figure 3-1) that, similar to Tsai et al. (2001) and Van Ommen et al. (1986a, b), involved oxidative dechlorination of PCP to benzoquinones via the corresponding semiquinones (also referred to as benzosemiquinones). The authors reported metabolites of PCP as TCHQ and TCpCAT. Both of these metabolites are thought to undergo oxidation to tetrachloro-1,4-benzosemiquinone (TCpSQ) and tetrachloro-1,2-benzosemiquinone (TCoSQ). The semiquinones subsequently undergo further oxidation to form the corresponding TCpBQ and tetrachloro-1,2-benzoquinone (TCoBQ).



Source: recreated from Lin et al. (2002).

Figure 3-1. Proposed PCP metabolism to quinols, benzosemiquinones, and benzoquinones.

3.2.1.4. *Excretion*

Uhl et al. (1986) measured elimination half-lives of 18–20 days in urine and 16 days in blood in human males orally administered 0.055, 0.061, 0.15, or 0.31 mg/kg PCP (dissolved in 40% ethanol). Urinary clearance was 1.25 mL/minute for free (unconjugated) PCP, while clearance for total PCP (free PCP and conjugated PCP-glucuronide) was shown to be very slow, only 0.07 mL/minute. Considering that >96% of the administered PCP was bound to plasma proteins in blood measurements, the authors suggested that bound PCP resulted in a relatively long elimination half-life and slow clearance.

Braun et al. (1979) reported elimination half-lives of 30 and 33 hours for plasma elimination and urinary excretion, respectively, in four human male subjects orally administered 0.1 mg/kg PCP (in 25 mL of water). Elimination was consistent with a first-order, one-compartment pharmacokinetic model. While plasma concentration peaked at 4 hours, peak urinary excretion occurred 42 hours after dosing; the delay in time was attributed to enterohepatic recirculation of PCP.

Braun et al. (1977) described a two-compartment open system model in rats administered PCP in corn oil, where the PCP elimination half-life of the rapid phase was 13–17 hours for both doses, while the slower phase was 33–40 hours at the 10 mg/kg dose and 121 hours for the 100 mg/kg dose in males. Females, however, did not show biphasic elimination at the 100 mg/kg dose; the rapid phase accounted for >90% elimination of the dose.

Larsen et al. (1972) reported that <0.04% of a 59 mg/kg oral dose of [^{14}C]PCP (99.5% purity; dissolved in olive oil) administered to male and female rats (strain not reported) was eliminated in expired air as $^{14}CO_2$ within 24 hours. After administration of 37–41 mg/kg, females excreted 41% of the radioactivity in urine within 16 hours, 50% within 24 hours, 65% within 72 hours, and 68% within 10 days. Fecal excretion accounted for 9.2–13.2% of the administered dose. Excretion showed a biphasic pattern, a rapid excretion phase during the first 24 hours and a slower phase thereafter.

Ahlborg et al. (1974) reported that NMRI mice and Sprague-Dawley rats excreted <50% of radioactivity in urine during the first 96 hours after oral administration of 25 mg/kg [^{14}C]PCP (dissolved in olive oil), with about twice as much appearing in the urine of rats compared with mice. About 70% of the radioactivity appeared in the urine after interperitoneal (i.p.) injection of 25 mg/kg. The radioactivity in the urine of mice and rats was 41 and 43% PCP and 24 and 5% TCHQ, respectively. Another metabolite, TCpCAT, made up 35% of the radioactivity in urine in the mouse and 52% in the rat. Because TCHQ inhibited β-glucuronidase activity, the degree of glucuronide conjugation could not be determined. However, boiling the urine with hydrochloric acid to release free metabolites from conjugates converted the entire radioactivity to PCP and TCHQ, with a nearly identical distribution of radioactivity between these metabolites (54 and 57% PCP and 46 and 43% TCHQ, respectively, in mice and rats).

Reigner et al. (1991) investigated PCP elimination in male Sprague-Dawley rats given 2.5 mg/kg PCP by either intravenous (i.v.) or oral (gavage) administration. The study authors reported biphasic plasma elimination with half-lives of 0.7 and 7.1 hours with i.v. administration. The data were fitted with an open two-compartment model. The areas under the curve (AUCs) were similar for i.v. and oral administration (96 and 94 μg-hours/mL, respectively). Total excretion was 68 and 62% and total urinary excretion was 58 and 52% of the PCP doses for i.v. and gavage administration, respectively. Total urinary TCHQ excretion was 31 and 27% of the PCP dose for i.v. and gavage administration, respectively. These data are similar in recovery to other studies in male rats (Braun et al., 1977), and in rats and mice (Ahlborg et al., 1974). However, the plasma elimination after oral administration (in corn oil) observed in male rats by Braun et al. (1977), while also following a biphasic pattern, showed much longer half-lives than those obtained by gavage administration in Reigner et al. (1991). Reigner et al. (1992c) reported that the elimination half-life in male $B6C3F_1$ mice was 5.8 hours. An analysis of metabolites revealed that only 8% of the administered PCP was excreted as parent compound. Yuan et al. (1994) noted sex differences in F344 rats with regard to elimination half-life (5.6 hours for males and 9.5 hours for females) and volume of distribution (0.13 L/kg for males and 0.19 L/kg for females). Bioavailability estimated from the AUC for i.v. injection and gavage administration was 100% at 9.5 mg/kg and 86% at 38 mg/kg PCP.

Rozman et al. (1982) demonstrated a significant effect of biliary excretion on disposition of orally administered PCP. Three male Rhesus monkeys equipped with a bile duct bypass were administered 50 mg/kg of [^{14}C]PCP by stomach intubation. During the first 24 hours, 21% of the administered dose was excreted into urine, 0.3% into feces, and 19% into bile. From day 2 to 7 after dosing, 35% of the administered dose was excreted into urine, 3% into feces, and 70% into bile. The monkeys received a second dose of 50 mg/kg [^{14}C]PCP, followed 24 hours later by 4% cholestyramine (binds phenols) in the diet for 6 days. Cumulative excretion of PCP into urine and bile was reduced to 5 and 52%, respectively, of the administered dose, whereas cumulative excretion into feces was increased to 54% of the dose. The data suggest that enterohepatic recirculation of PCP plays a major role in urinary excretion of the compound. In Rhesus monkeys administered a single 10 mg/kg dose of [^{14}C]PCP, the plasma elimination half-lives ranged from 72 to 84 hours, and the urinary excretion half-life was 41 hours for males and 92 hours for females (Braun and Sauerhoff, 1976). Urinary excretion accounted for 69–78% of the administered dose and feces for 12–24%. Unlike humans and rats, all of the PCP eliminated in the urine of monkeys was unchanged parent compound (Braun and Sauerhoff, 1976). The Rozman et al. (1982) data are not directly comparable with those obtained by Braun and Sauerhoff (1976) because of the bile duct bypass; however, a relative correlation with the excretion pattern is indicated.

Deichmann et al. (1942) administered 0.1% PCP sodium salt (equivalent to 3 mg/kg; in feed) to rabbits repeatedly for 90 successive (except Sundays) doses and about 92% of the dose

was recovered in urine, feces, and tissues combined (~71% in urine and feces) within the first 24 hours, and elimination from the blood was almost complete within 4 days after dosing. The largest fractional tissue dose was recovered from muscle, bone, and skin; however, 0.7–2% of the dose was recovered in the liver. Deichmann et al. (1942) also showed that rabbits orally administered 25 and 50 mg/kg PCP sodium salt (in feed) excreted 64–70 and 49–56% of the dose in urine and feces, respectively, within 7 and 12 days.

The absorption and elimination half-lives and the maximum plasma concentrations for orally administered PCP in rats, mice, and monkeys are summarized in Table 3-1. Human data from Braun et al. (1979) are also included for comparison. The kinetics of orally administered PCP, for all of the species studied, are consistent with a one- or two-compartment open model exhibiting first order kinetics. Based on the available data, the toxicokinetics of PCP in humans may be more similar to those of rats and mice than Rhesus monkeys.

Table 3-1. Summary of some toxicokinetic parameters in rats, monkeys, and humans for orally administered PCP

Species	Absorption $t_{1/2}$ (hrs)	Plasma T_{max} (hrs)	Elimination $t_{1/2}$ (hrs)	Process description	Reference
Human	1.3	4	30–33	1st order, one compartment	Braun et al. (1979)
Rhesus monkey	1.8–3.7	12–24	72–84	One compartment, open	Braun and Sauerhoff (1976)
Rat	–	4–6	13–17 (fast) 33–40 (slow)	Two compartment, open	Braun et al. (1977)
Rat	1.3	2–4	5.6–9.5	1st order, one compartment	Yuan et al. (1994)
Mouse	0.6	1.5	5.8	1st order, one compartment, open	Reigner et al. (1992c)

3.2.2. Inhalation Studies

PCP inhaled by rats showed rapid uptake from the respiratory tract and excretion from the body. Hoben et al. (1976a) exposed Sprague-Dawley rats to PCP aerosols at a dose of 5.7 mg/kg for 20 minutes and measured PCP at 0, 6, 12, 24, 48, and 72 hours after exposure. Between 70 and 75% of the PCP could be accounted for as unmetabolized PCP within the first 24 hours; the highest level was in urine >liver = plasma >lungs. PCP in lung and liver showed a steady decrease throughout the study; plasma levels showed a steady decrease after a peak at 6 hours; and urine showed a steady decrease after 24 hours. The estimated half-life was 24 hours, and there was no evidence of accumulation or tissue binding.

Rats exposed to PCP aerosols repeatedly for 20 minutes/day for 5 days showed only a slight net increase in lung and plasma levels immediately after the second exposure with no net increase in liver levels (Hoben et al., 1976a). Twenty-four hours after each exposure, lung, liver, and plasma levels were lower but urine levels increased, suggesting that increased urinary excretion may explain the lack of accumulation of body burden upon repeated exposures.

However, the study authors noted that increased urinary excretion did not account entirely for the lack of accumulation; they also concluded that metabolism was likely involved.

3.2.3. Dermal Studies

Bevenue et al. (1967) reported on a case in which a man immersed his hands for 10 minutes in a solution containing PCP (0.4%). The initial urinary concentration measured 2 days after the incident was 236 ppb. The urinary level declined to 34% of the initial concentration by day 3, 20% after two weeks, 27% after three weeks, 10% after 1 month, and 7% after 2 months. This report shows that PCP is rapidly absorbed through the skin. Elimination was rapid during the first 4 days and proceeded more slowly thereafter. Because elimination is initially rapid, the concentration of PCP in urine was likely much higher during the first 24 hours after exposure than after 2 days.

Wester et al. (1993) reported on the absorption of PCP through the skin of female Rhesus monkeys. PCP-contaminated soil (17 ppm [^{14}C]PCP) or [^{14}C]PCP in acetone was applied topically at a concentration of 0.7 or 0.8 μg/cm^2 of skin, respectively, for 24 hours. The percutaneous absorption levels were determined by comparing the urinary excretion levels of [^{14}C]PCP following either topical or i.v. administration. The measured percent dose peaked on day 1 for topical and on day 2 for i.v. application, and exhibited a steady decline for approximately 7 days followed by relatively level daily excretion rates. Over the 14-day collection period, 45, 11, and 13% of the applied dose was excreted in the urine following i.v., topical-soil, and topical-acetone applications, respectively. Percutaneous absorption was similar for both vehicles with 24 and 29% of the applied dose recovered for soil and acetone, respectively. The [^{14}C] half-life for excretion was 4.5 days after i.v. administration. Similarly, the topical administration of PCP, either in soil or acetone, also indicated [^{14}C] half-lives of 4.5 days. The efficient absorption of PCP from skin is indicative of high bioavailability. Similar to that observed in humans by Bevenue et al. (1967), the relatively long half-life of PCP observed in the dermal application increases the potential for biological interaction.

3.2.4. Other Studies

Jakobson and Yllner (1971) exposed mice to 1 or 0.5 mg [^{14}C]PCP via i.p. injection. The investigators reported the greatest amount of PCP distributed in the mice was found in the liver, intestines, and stomach. Lesser amounts of the dose were found in the heart, kidney, and brain. Within 96 hours after injection, 72–83% of the dose was excreted in urine and 3.8–7.8% was excreted in feces; the remainder of the dose was found in specific organs and the carcass. Rapid absorption and excretion of PCP was exhibited by the appearance of 45–60% of the dose in urine within the first 24 hours. The authors found that approximately 30% of the PCP measured in the urine of mice administered 1 or 0.5 mg [^{14}C]PCP was unmetabolized, 7–9% was bound but released by acid treatment, and 15–26% was the metabolite TCHQ.

3.3. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

No physiologically based pharmacokinetic (PBPK) models for the oral or inhalation routes of exposure in humans or animals are available.

4. HAZARD IDENTIFICATION

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

This section reviews the available evidence of health effects in humans resulting from exposure to PCP, focusing on carcinogenicity, acute toxicity, and neurological, developmental, and reproductive effects of chronic exposures.

4.1.1. Studies of Cancer Risk

4.1.1.1. *Case Reports and Identification of Studies for Evaluation of Cancer Risk*

Significant production of PCP began in the 1930s. The earliest report of cancer was about 40 years later when Jirasek et al. (1976 [in German]) examined the condition of 80 factory workers. In addition to porphyria and other serious conditions, two workers had died of bronchogenic carcinoma, which the authors attributed to contamination from 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD). Other case reports published around this time described non-Hodgkin's lymphoma among PCP manufacturing workers (Bishop and Jones, 1981) and Hodgkin's disease in employees of a fence installation company who experienced high exposure to PCP through the application of the wood preserving solution (Greene et al., 1978).

Several epidemiologic studies conducted in the 1970s and 1980s examined cancer risk in relation to broad occupational groups (e.g., wood workers, agricultural, and forestry workers) (Pearce et al., 1985; Greene et al., 1978; Brinton et al., 1977). Some subsequent studies focused on specific workplaces and jobs with known exposures to PCP (e.g., PCP manufacturing plants, sawmills in which industrial hygiene assessments had been made). Other studies were conducted in general population samples and used exposure assessments that attempted to distinguish specific exposures, which sometimes included PCP, within broad occupational groups (e.g., specific farming-related activities or exposures).

Studies with PCP-specific data are described in the subsequent section. Some studies provide data pertaining to exposure to chlorophenols. These studies were included in this summary when specific information was presented in the report pertaining to PCP (for example, results for specific jobs that would be likely to have used PCP, rather than other chlorophenols). Studies that presented data only for a combined exposure (e.g., chlorophenols, or chlorophenols and phenoxy herbicides) are not included (Richardson et al., 2008; 't Mannetje et al., 2005; Mirabelli et al., 2000; Garabedian et al., 1999; Hooiveld et al., 1998; Hoppin et al., 1998; Kogevinas et al., 1997; Ott et al., 1997; Mikoczy et al., 1996; Johnson et al., 1990). A cohort study of sawmill workers in Finland and a study of cancer incidence in the area surrounding a mill were identified but not included (Lampi et al., 1992; Jäppinen et al., 1989) because the chlorophenol exposure was primarily to TCP, with PCP representing <10% of the chlorophenol exposure. Two papers describing studies of surveys of exposed workers contained some

information pertaining to cancer mortality. Cheng et al. (1993) examined a small, relatively young cohort (n = 144) from a PCP manufacturing plant in China, where a total of 3 deaths occurred during follow-up, and Gilbert et al. (1990) examined mortality rates in 125 wood workers in Hawaii, where a total of 6 deaths occurred. The mortality data in these studies were very limited (cohort size <200; lack of information pertaining to follow-up and other methodologic details, limited comparison data, particularly with respect to cancer-specific mortality); therefore these studies are not included in this section.

The studies summarized in this review include three cohort studies of workers occupationally exposed to PCP (plywood mill workers, PCP manufacturing workers, and sawmill workers), and 12-case control studies (4 of which were summarized in a meta-analysis) of lymphoma, soft tissue sarcoma, or multiple myeloma. When two papers on the same cohort were available, the results from the longer period of follow-up are presented in the summary. Information from earlier reports is used when these reports contain more details regarding working conditions, study design, and exposure assessment. The study setting, methods (including exposure assessment techniques), results pertaining to incidence or mortality from specific cancers, and a brief summary of primary strengths and limitations are provided for each selected study. The limited data pertaining to liver cancer are presented because the liver is a primary site seen in the mouse studies (NTP, 1989). Other data emphasized in this summary relate to lymphatic and hematopoietic cancers, and soft tissue sarcoma, because of the quantity of data and interest in this area. The description of individual studies is followed by a summary of the evidence available from all studies reviewed relating to specific types of cancer.

4.1.1.2. *Cohort Studies*

Three cohort studies of workers exposed to PCP have been conducted, and in two of these, a PCP-specific exposure measure was developed and used in the analysis (Table 4-1). Ramlow et al. (1996) examined the mortality risk in a cohort of 770 male workers at a large U.S. chemical manufacturing plant (Dow Chemical Company, Michigan Division) that manufactured PCP from the late 1930s to 1980. This cohort was a subset of a larger cohort of workers in departments with potential for exposure to tPCP. Exposure to dioxins, primarily hexa-, hepta-, and octa-chlorinated dibenzodioxins and dibenzofurans also occurred within this cohort (Ott et al., 1997). Men who were employed at the Michigan plant between 1937 and 1980 were included in the study. Follow-up time was calculated through 1989. The mean durations of work or exposure were not reported, although the mean duration of follow-up was 26.1 years.

Table 4-1. Summary of cohort studies of cancer risk and PCP exposure, by specificity of exposure assessment

Reference, cohort, location	Total number, duration of work, and follow-up	Inclusion criteria	Exposure assessment	Outcome assessment	Results–PCP risk[a]
Pentachlorophenol, specific exposure					
Ramlow et al. (1996), Dow manufacturing plant, United States (Michigan)	n = 770 men mean duration: not reported mean follow-up: 26.1 years	Worked sometime between 1937 and 1980 in a relevant department	Work history (job records) and industrial hygiene assessment; developed exposure intensity and cumulative exposure scores for PCP and dioxins[b]	Death certificate (underlying cause)	Elevated risk of lymphatic cancer mortality, particularly at higher intensity exposures (RR 2.58 (95% CI 0.98–6.8)[c]; similar associations seen with measures of other dioxins
Demers et al. (2006) Hertzman et al. (1997) Heacock et al. (2000), sawmill workers, Canada (British Columbia)	n = 23,829 men mean duration: 9.8 years mean follow-up: 24.5 years	Worked at least 1 year (or 260 days total) between 1950 and 1985	Work history (job records) and industrial hygiene assessment; developed cumulative exposure scores for PCP and TCP	Death certificate (underlying cause); Cancer registry (incidence)	Elevated risk of non-Hodgkin's lymphoma and multiple myeloma incidence and mortality; evidence of exposure-effect response; weaker or no risk seen with TCP (see Table 4-2). No increased risk of childhood cancer in offspring of workers
Pentachlorophenol, nonspecific exposure					
Robinson et al. (1987), plywood mill workers, United States (Pacific Northwest)	n = 2,283 men mean duration: not reported mean follow-up: 25.2 years	Worked at least 1 year between 1945 and 1955	Work history (job records); subgroup analysis of 818 workers known to have worked in areas with PCP or formaldehyde exposure	Death certificate (underlying cause)	Elevated risk of lymphatic and hematopoietic cancer mortality (SMR = 1.56 (95% CI 0.90–2.52); stronger when considering latency and duration, and when limited to subgroup with PCP or formaldehyde exposure

[a]Results are described as "elevated" if standardized mortality ratio (SMR) was around 1.5 or higher, regardless of the precision of the estimate or power of the statistical test; more detailed information on the results is presented in the text.
[b]2,3,7,8-TCDD and the hexachlorinated to octachlorinated dioxin ratio.
[c]For the category of "other and unspecified lymphopoietic cancers" (now classified as multiple myeloma and non-Hodgkin's lymphoma).

Potential for exposure to PCP was assessed by evaluating available industrial hygiene data, including some quantitative environmental and personal breathing zone PCP measurements in conjunction with detailed employment records with information on job title and location. Potential exposures for each job held by cohort members were assigned an estimated exposure

intensity score on a scale of 1 (low) to 3 (high). An estimated cumulative exposure index was calculated for each subject by multiplying duration for each job by the estimated exposure intensity for the job and summing across jobs. The cumulative exposure scores were <1 for 338 (44%), 1–2.9 for 169 (22%), 3–4.9 for 74 (10%), 5–9.9 for 83 (11%) and ≥10 for 106 (14%) of the workers. A similar process was used to estimate cumulative exposure to 2,3,7,8-TCDD and the hexachlorinated to octachlorinated dioxin ratio. Standardized mortality rates (SMRs) were calculated comparing age- and period-specific mortality rates in the cohort and the U.S. white male population. The cumulative exposure metric was used with an internal reference group, allowing for examination of exposure-response in analyses estimating relative risk (RR) controlling for age, period of employment, and general employment status (hourly vs. salaried).

Mortality risk for all causes of cancer was not elevated (standardized mortality ratio [SMR] 0.95, 95% confidence interval [CI] 0.71–1.25), and there were no reported cases of mortality due to liver cancer, soft tissue sarcoma, or Hodgkin's disease. The SMR was 2.31 (95% CI 0.48–6.7) for kidney cancer (International Classification of Disease [ICD]-8th revision codes 189; three cases), with the highest risk seen in the high-exposure group (defined as cumulative exposure ≥10; relative risk (RR) 4.16 (95% CI 1.43–12.09; trend p-value 0.03). An elevated kidney cancer mortality risk was also seen with increased dioxin measures in this cohort (for TCDD, trend p-value = 0.04; for hexachlorinated to octachlorinated dioxin ratio, trend p-value = 0.02). The SMR for all lymphopoietic cancers (ICD-8th revision codes 200-209; seven cases) was 1.4 (95% CI 0.56–2.88). This latter observation was driven by the results for the "other and unspecified lymphopoietic cancers" (ICD-8th revision codes 200, 202–203, 209; five cases), with an SMR of 2.0 (95% CI 0.65–4.7). Two of these cases were multiple myeloma, and three would now be classified as non-Hodgkin's lymphoma. Similar results were seen in analyses using a 15 year latency period. In the exposure-response analysis, the RR in the high-exposure group (defined as cumulative exposure ≥ 1) compared with the no-exposure group was 1.91 (95% CI 0.86–4.24, trend *p*-value 0.23) for all lymphopoietic cancers, and 2.58 (95% CI 0.98–6.8, trend *p*-value 0.08) for other and unspecified lymphopoietic cancers. There was some indication of an increased risk of lymphopoietic cancer with the other dioxin measures, primarily seen in the "very low" or "low" exposure groups.

The exposure assessment methodology, allowing for the analysis of PCP and various forms of dioxins exposure, is the primary strength of this study. The cumulative exposure metric used in the analysis was based on work duration data in conjunction with a semiquantitative intensity score for specific jobs; the semiquantitative nature of this measure presents challenges to its use in dose-response modeling for risk assessment. It is a relatively small cohort, however, resulting in limited power to assess associations with relatively rare cancers, including the various forms of lymphomas, soft tissue sarcoma, and liver cancer. Other limitations of this study are its use of mortality, rather than incidence data, and the difficulty in separating the effects of exposures to different dioxins that occurred as part of the production process.

Hertzman et al. (1997) conducted a large cohort study of male sawmill workers from 14 mills in Canada (British Columbia), and this study was recently updated by Demers et al. (2006). Sodium salts of PCP and TCP were used as fungicides in 11 of these mills from 1950 to 1990. Workers from the mills that did not use the fungicides (n = 2,658 in Hertzman et al., 1997; sample size not specified in Demers et al., 2006) were included in the unexposed group in the exposure-response analyses. The updated study includes 26,487 men who had worked at least 1 year (or 260 days total) between 1950 and 1995. Record linkage through the provincial and national death files and cancer incidence registries were used to assess mortality (from first employment through 1995) and cancer incidence (from 1969, when the provincial cancer registry began, through 1995) (Demers et al., 2006). The mean duration of work in the mills was not given in the 2006 update by Demers et al. (2006), but in the earlier report of outcomes through 1989 (Hertzman et al., 1997), the mean duration of employment was 9.8 years, and the mean duration of follow-up was 24.5 years. Approximately 4% of the cohort was lost to follow-up, and these individuals were censored at date of last employment.

Plant records were available to determine work histories for study cohort members, including duration of work within different job titles. Representative exposures were determined for three or four time periods for each mill. Historical exposure measurements had not been made, so a retrospective exposure assessment was developed based on interviews with senior workers (≥5 years of experience) at each mill (9–20 workers for each time period; mean of 15 years of experience). This process was compared, for current exposures, to urinary measurements, with correlation coefficients of 0.76 and 0.72 in two different sampling periods (summer and fall) (Hertzman et al., 1988). Because only one sample was collected in each period, day to day variation in job activities, and thus exposures, would not be captured by the urine measure; the authors indicate that additional samples would likely result in increased correlation coefficients. The validity of this method was also demonstrated in comparison with a method based on an industrial hygienist assessment (Teschke et al., 1996, 1989).

Information from the senior workers was used to develop a cumulative dermal chlorophenol exposure score, calculated for each worker by summing, across all jobs, the product of the job title specific exposure score and the length of employment in that job. One exposure year was defined as 2,000 hours of dermal contact. Records from each mill were used to determine the specific chlorophenol content of the fungicides used at specific time periods. In general, TCP was using increasingly in place of PCP after 1965. This information was used to develop PCP- and TCP-specific exposures scores. The correlation between the estimated PCP and TCP exposures was 0.45 (Demers et al., 2006).

Soft tissue sarcoma is difficult to ascertain accurately without review of the available histological information. Demers et al. (2006) did not include an analysis of soft tissue cancer mortality risk (which would have had to rely only on death certificate classification data). The

authors based the analysis of incident soft tissue sarcoma on cancer registry data pertaining to site (connective tissue) and histology.

SMR and standardized incidence ratios (SIRs) were calculated using reference rates based on data for the province of British Columbia. Analyses using the quantitative exposure measure used workers in the cohort with <1 exposure-year as the internal referent group. All analyses were adjusted for age, calendar period, and race.

There was no increased risk with respect to cancer-related mortality (SMR 1.00, 95% CI 0.95–1.05) or incidences of all cancers (SIR 0.99, 95% CI 0.95–1.04) in the cohort of sawmill workers. In the analyses of PCP exposure, there was evidence of an exposure effect for non-Hodgkin's lymphoma and multiple myeloma in the mortality and in the incidence analyses (Table 4-2). The risk of non-Hodgkin's lymphoma in relation to TCP was similar to or somewhat smaller than for PCP, and no association was seen between TCP exposure and multiple myeloma. The number of incident cases of soft tissue sarcoma was small (n = 23), and lower risks of this cancer were seen in the higher exposure groups for PCP and for TCP. There was some evidence of an increased risk of kidney cancer incidence or mortality for PCP and TCP exposures (Table 4-2). Liver cancer, a relatively rare cancer, was associated with PCP exposure, but the sparseness of data did not allow assessment at the highest exposure level (>5 exposure years). Consideration of a 10- or 20-year exposure lag period had little effect on the risks seen with respect to PCP exposure and risk of non-Hodgkin's lymphoma, multiple myeloma, and kidney cancer incidence. The 20-year lag resulted in a reduction in the number of liver cancer cases in the exposed categories from 18 to 2, and thus the pattern of increased risk was no longer seen. Friesen et al. (2007) examined these data using different models and exposure metrics, and using the best-fitting lagging period as seen in the Demers et al. (2006) analysis. The results of Friesen et al. (2007) study indicates that for non-Hodgkin's lymphoma and kidney cancer the PCP risk was stronger than that seen for TCP or total chlorophenols.

Table 4-2. Cancer mortality and incidence risk in relation to estimated PCP exposure in sawmill workers, British Columbia, Canada[a]

		Pentachlorophenol exposure						Tetrachlorophenol exposure					
		Mortality			Incidence			Mortality			Incidence		
Cancer	Exposure-years	Obs	RR	95% CI	Obs	RR	95% CI	Obs	RR	95% CI	Obs	RR	95% CI
Non-Hodgkin's lymphoma	<1	15	1.0	(referent)	38	1.0	(referent)	29	1.0	(referent)	50	1.0	(referent)
	1–2	6	1.21	0.46–3.2	13	1.33	0.70–2.5	5	0.93	0.36–2.43	11	0.91	0.47–1.75
	2–5	18	2.44	1.2–5.1	24	1.88	1.1–3.3	13	1.96	0.99–3.89	20	1.34	0.80–2.26
	5+	10	1.77	0.75–4.2	17	1.71	0.91–3.2	2	0.63	0.15–2.69	11	1.54	0.79–2.99
	(trend[b])			(0.03)			(0.06)			(0.44)			(0.14)
Multiple myeloma	<1	4	1.0	(referent)	6	1.0	(referent)	15	1.0	(referent)	15	1.0	(referent)
	1–2	5	3.30	0.87–12.5	4	2.09	0.57–7.6	0	0.00		1	0.27	0.04–2.04
	2–5	4	1.58	0.38–6.6	4	1.30	0.34–5.0	4	0.94	0.31–2.91	5	1.06	0.38–2.94
	5+	10	4.80	1.4–16.5	11	4.18	1.4–12.9	4	1.84	0.59–5.78	4	1.80	0.58–5.60
	(trend[b])			(0.03)			(0.02)			(0.55)			(0.48)
Soft tissue sarcoma[c]	<1				18	1.0	(referent)				16	1.0	(referent)
	1–2				3	0.64	0.18–2.2				3	0.77	0.23–2.66
	2–5				2	0.18	0.04–0.85				4	0.66	0.22–1.99
	5+				0						0		
	(trend[b])						(0.11)						(0.43)
Kidney	<1	15	1.0	(referent)	32	1.0	(referent)	25	1.0	(referent)	47	1.0	(referent)
	1–2	6	1.33	0.51–3.5	9	1.03	0.49–2.2	5	0.94	0.36–2.46	6	0.55	0.23–1.28
	2–5	17	2.59	1.22–5.5	22	1.79	0.99–3.2	14	2.09	1.07–4.08	14	1.01	0.56–1.84
	5+	12	2.30	1.00–5.3	16	1.66	0.85–3.2	6	1.87	0.75–4.67	12	1.80	0.94–3.43
	(trend[b])			(0.02)			(0.07)			(0.04)			(0.31)
Liver	<1	4	1.0	(referent)	3	1.0	(referent)	4	1.0	(referent)	11	1.0	(referent)
	1–2	5	3.46	0.91–13.2	4	4.09	0.89–18.8	8	0.95	0.38–2.4	7	2.65	1.03–6.85
	2–5	8	3.72	1.04–13.3	12	8.47	2.2–32.4				3	0.52	0.14–1.88
	5+	5	2.53	0.61–10.4	2	1.41	0.21–9.2				0		
	(trend[b])			(0.10)			(0.18)						(0.58)

[a] Obs = number of observed cases. Analyses based on Poisson regression using the lowest exposure group as the referent group, adjusting for age and time period.
[b] Trend *p*-value.
[c] The authors used histology data for the classification of soft tissue sarcoma, so mortality data (from death certificates, without detailed histology information) was not analyzed for this disease.

Source: Demers et al. (2006).

Heacock et al. (2000) examined risk of childhood cancer among the offspring of the male workers in the British Columbia sawmill workers cohort. (An additional study by Dimich-Ward et al. (1996), based on this cohort, of pregnancy outcomes, including prematurity, stillbirths, and congenital anomalies, is discussed in Section 4.1.2.4, Studies of Reproductive Outcomes.) Marriage and birth records were linked to identify 19,675 children born to these fathers between 1952 and 1988. Forty incident childhood cancers were identified within these children (with follow-up through age 19 years) through the linking of these birth records to the provincial cancer registry. Eleven of the cancers were leukemias, nine were brain cancers, and four were lymphomas. The incidence rates were similar to those expected based on sex, age, and calendar year standardized rates, with a SIR of 1.0 (95% CI 0.7–1.4) for all cancers, 1.0 (95% CI 0.5–1.8) for leukemia, and 1.3 (95% CI 0.6–2.5) for brain cancer.

The large size and long follow-up period are important strengths of the British Columbia sawmill cohort studies (Demers et al., 2006; Heacock et al., 2000; Hertzman et al., 1997), but even with this size, there is limited statistical power to estimate precise associations with relatively rare cancers such as liver cancer and soft tissue sarcoma. Other strengths of the study include the detailed exposure assessment (for PCP and TCP), completeness of follow-up, and analysis of cancer incidence (through the coverage of the population-based cancer registry) in addition to mortality. The observed associations are not likely to be explained by confounding: common behaviors, such as smoking and use of alcohol, have not been associated with the types of cancers that were associated with PCP exposure in this study (non-Hodgkin's lymphoma, multiple myeloma); the use of an internal comparison group for the analyses using the exposure measures reduces the likelihood of potential confounders affecting the results, and the difference in the patterns with respect to cancer risks seen between PCP and TCP and between PCP and dioxins also argues against a role of other occupational exposures or contaminants of PCP as an explanation for the observed associations. (See Section 4.1.1.4, General Issues—Interpretation of the Epidemiologic Studies, for additional discussion of this issue.) No information is provided, however, about the effect of adjustment for TCP exposure on the PCP results. Since the correlation between the two measures is relatively low (r = 0.45), and for many of the cancers of interest the PCP associations are stronger than those seen with TCP, it is unlikely that this adjustment would greatly attenuate the observed associations with PCP. Additional analyses by the study authors could address this issue, although the relatively small number of observed cases for specific cancers of interest is likely to be a limitation of this kind of analysis.

Robinson et al. (1987) examined mortality in a cohort of 2,283 male plywood mill workers employed at four softwood plywood mills in Washington and Oregon (Table 4-1). Protein glues were used to join the veneer plies, and PCP was often added to the glues as a mold preventative. PCP was also added to oils used as mold release agents during finishing of the plywood panels. Other exposures in the various jobs at the mills included wood dust, wood volatiles, formaldehyde, and carbon disulfide. One subgroup analysis was conducted of workers

(n = 818) who had worked in areas with PCP or formaldehyde exposures. There was no increased risk of mortality for all sites of cancer (SMR 0.70). Data pertaining to cancer of the liver were not reported. The SMR was 1.56 (95% CI 0.90–2.52) for lymphatic and hematopoietic cancers (ICD-7th edition codes, ICD, 200–203, 205; based on 12 cases) and 0.86 (95% CI not reported) for leukemia (ICD code 204, based on 5 cases). For lymphatic and hematopoietic cancers, this increased risk was stronger when using a latency period of 20 years (SMR of 1.95) and when the analysis was limited to duration of employment of >20 years (SMR of 2.50). The risk of lymphopoietic cancer was also stronger in the subgroup of workers designated as exposed to PCP or formaldehyde (SMR 2.50 [95% CI 0.61–6.46] for lymphatic cancer and 3.33 [95% CI 0.59–10.5] for Hodgkin's lymphoma). A major limitation of this study is that there is no analysis specifically focused on PCP exposure, and the co-exposure with formaldehyde is particularly relevant for the lymphopoietic cancers.

4.1.1.3. *Case-Control Studies of Specific Cancers and Pentachlorophenol*

Six case-control studies have reported data pertaining to PCP exposure in relation to risk of lymphoma (Table 4-3). Three of these studies also included analyses of risk of soft tissue sarcoma, and five additional case-control studies of soft tissue sarcoma (four of which were summarized in the meta-analysis by Hardell et al. [1995]) are also available (Table 4-4). Case-control studies of multiple myeloma (Pearce et al., 1986a) and of childhood and young adult cancers (Ali et al., 2004) are also included in this summary.

Table 4-3. Summary of case-control studies of lymphoma[a] risk and PCP exposure

Reference, location, demographic data, diagnosis years	Cases (n, source), Controls (n, source)	Source of exposure data	Results[b]
Detailed PCP assessment			
Kogevinas et al. (1995), Europe[c]	32 cases (death certificates for all countries; cancer registries for 7 countries), 158 controls (nested case-control study within cohort study of exposed workers[c])	Company records and industrial hygienist review	PCPs: OR[b] = 2.75 (95% CI 0.45– 17.0) High PCPs: OR = 4.19 (95% CI 0.59–29.6)
Hardell et al. (1994, 1981), Sweden, men, age 25–85 years, 1974 to 1978	105 cases (hospital records) (62% deceased); 355 population controls (matched by vital status)	Self-administered questionnaire with follow-up phone interview if needed[d]	High (more than 1 week continuously or 1 month total) exposure to PCPs: OR = 8.8 (95% CI 3.4–24)
Hardell and Eriksson (1999), Sweden, men, age >25 years, 1987 to 1990	442 cases (cancer registry) (43% deceased) 741 population controls (matched by vital status)	Self-administered questionnaire with follow-up phone interview if needed[d]	PCPs: OR 1.2 (95% CI 0.7–1.8)
Limited PCP assessment			
Pearce et al. (1986b), New Zealand, men, age <70 years, 1977-1981	83 cases (cancer registry) (% deceased not specified) 168 cancer controls (% deceased not specified), and 228 population controls	Structured interview[d]	Chlorophenols: OR = 1.3 (95% CI 0.6–2.7) Fencing work: OR = 2.0 (95% CI 1.3–3.01)
Woods et al. (1987), United States - Washington, men, age 20–79 years, 1983 to 1985	576 cases (cancer registry) (30% deceased) 694 population controls (32% deceased)	Structured interview[d]	Chlorophenols: OR = 0.99 (95% CI 0.8–1.2) Increased risk (OR >1.5) for wood preservers and chlorophenols manufacturers but not for lumber grader (OR = 0.94)
Smith and Christophers (1992), Australia, men, age ≥30 years, 1976 to 1980	52 cases (cancer registry), 52 cancer controls and 52 population controls Deceased cases and controls excluded	Structured interview	Chlorophenols: OR = 1.4 (95% CI 0.3–6.1) Four cases and four controls (one population and three cancer controls) had definite PCP exposure

[a]Non-Hodgkin's lymphoma except for Smith and Christophers (1992), which includes non-Hodgkin's and Hodgkin's
[b]OR = Odds ratio
[c]Twenty cohorts from 10 countries workers; total n = 13,898; workers exposed to phenoxy herbicides or chlorophenols. The follow-up period varied among the cohorts: follow-up began between 1942 and 1973 and ended between 1987 and 1992 (Kogevinas et al., 1997).
[d]Proxies included for deceased cases and controls.

Table 4-4. Summary of case-control studies of soft tissue sarcoma risk and PCP exposure

Reference, location, Demographics, diagnosis years	Cases (n, source), Controls (n, source)	Source of exposure data	Results
Detailed PCP assessment			
Kogevinas et al. (1995), Europe [a]	12 cases (death certificates for all countries; cancer registries for 7 countries), 44 controls (nested case-control study within cohort study of exposed workers)	Company records and industrial hygienist review	PCPs: no exposed cases or controls
Hardell et al. (1995) meta-analysis of 4 studies[b], Sweden, men, ages 25–80 years, 1970-1983	434 cases (hospital records; cancer registry), 948 population controls	Self-administered questionnaire with follow-up phone interview if needed[c]	High (more than 1 week continuously or 1 month total) exposure to PCPs: OR = 2.8 (95% CI 1.5–5.4)
Limited PCP assessment			
Smith et al. (1984), New Zealand, males, age 20–80 years, 1976 to 1980	82 cases (cancer registry) (% deceased not specified) 92 cancer controls (% deceased not specified)	Structured interview[c]	Chlorophenols: OR = 1.5 (95% CI 0.5–4.5) Variable results (ORs = 0.7–1.9) for fencing and sawmill/timber merchant jobs
Woods et al. (1987), United States - Washington, men, age 20–79 years, 1983 to 1985	128 cases (cancer registry) (24% deceased) 694 population controls (32% deceased)	Structured interview[c]	Chlorophenols: OR = 0.99 (95% CI 0.7–1.5) Lumber grader: OR = 2.7 (95% CI 1.1–6.4) Variable results (ORs = 0.79–4.8) for other "high," "medium," or "low" exposure jobs
Smith and Christophers (1992), Australia, men, age ≥30 years, 1976 to 1980	30 cases (cancer registry), 30 cancer controls and 30 population controls Excludes deceased cases and controls	Structured interview	Chlorophenols ≥1 day: 0 cases with this exposure 0 cases and 2 controls (1 population and 1 cancer control) had definite PCP exposure

[a] Twenty cohorts from 10 countries workers; total n = 13,898; workers exposed to phenoxy herbicides or chlorophenols. The follow-up period varied among the cohorts: follow-up began between 1942 and 1973 and ended between 1987 and 1992 (Kogevinas et al., 1997).
[b] The four case-control studies are described in Eriksson et al., 1990; Hardell and Eriksson, 1988; Eriksson et al., 1981; and Hardell and Sandstrom, 1979. More detailed information the individual studies is shown in Table 4-5.
[c] Proxies included for deceased cases and controls.

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Case-control studies of lymphoma. Three case-control studies provided data pertaining to risk of non-Hodgkin's lymphoma in relation to PCP using relatively detailed exposure data (Table 4-3). Kogevinas et al. (1995) conducted a nested case-control study of non-Hodgkin's lymphoma in the large, international cohort of 13,989 workers exposed to phenoxy herbicides or chlorophenols assembled from 20 cohorts in 10 countries. Job records and company records pertaining to chemicals used during specific processes were used by three industrial hygienists to evaluate exposure to 21 specific chemicals (phenoxy herbicides, chlorophenols, polychlorinated dibenzodioxins, furans, and process chemicals and raw materials). Cases of non-Hodgkin's lymphoma (n = 32) were identified by review of death certificates (underlying and contributing causes of death) for all countries, and review of cancer registries for the seven countries that had national registries. Five controls were selected per case from within the cohort, matched by age, sex, and country, for a total of 158 controls. The estimated associations in this study are relatively imprecise, given the small size, but there is evidence of an association with any PCP exposure (odds ratio [OR] = 2.75, 95% CI 0.45–17.0) and specifically with the high exposure, cumulative exposure category (OR = 4.19, 95% CI 0.59–29.6). Increased risks were not observed (i.e., ORs between 0.65 and 1.03) with the other specific chlorophenols examined (2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-TCP), and the associations seen with phenoxy herbicides and dioxins were also weaker than those seen with PCP (OR = 1.84 for any dioxin or furan, 1.93 for 2,3,7,8-TCDD). Although this is a small study, it is based within a large cohort for which detailed exposure assessments for a variety of compounds are available.

Hardell et al. (1994, 1981) conducted a population-based case-control study of non-Hodgkin's lymphoma in men ages 25–85 years in Umeå, Sweden. Cases (n = 105) with a diagnosis occurring between 1974 to 1978 were identified through hospital records; 355 population controls were identified through a population registry (for matching to living cases) and the national death registry (for matching to deceased cases); individual-matching, rather than frequency matching, was used. A self-administered questionnaire with follow-up phone interview if needed was used to obtained detailed information pertaining to work history, including information on specific jobs, and exposures. Next-of-kin proxy respondents were used for deceased cases and controls. The questionnaire information was used to create an exposure measure for specific chemicals, including chlorophenols and PCPs. The follow-up interview and the evaluation of the questionnaire information was conducted without knowledge of case or control status of the respondent. Exposures in the 5 years immediately preceding diagnosis (or a corresponding reference year for controls) were excluded to account for a minimum latency period. High exposure was defined as either 1 week or more of continuous exposure, or exposure for at least 1 month in total; those exposures less than this were considered low-grade. A strong association (OR = 8.8, 95% CI, 3.4–24) was observed between high exposure to PCP (the predominant chlorophenol used in this area) and risk of non-Hodgkin's lymphoma.

A subsequent case-control study of non-Hodgkin's lymphoma covering a larger study area (7 counties in northern and in mid-Sweden) was conducted by Hardell and Eriksson (1999). This study was limited to men ages ≥25 years diagnosed between 1987 and 1990. Procedures for case identification and recruitment of controls from the National Population Registry or, for matching to deceased cases the National Registry for Causes of Death, were similar to those used by Hardell et al. (1981, 1994). The study included 404 cases (43% deceased) and 741 controls. Exposure information was collected with a self-administered mailed questionnaire with follow-up phone interview if needed for clarification. Next-of-kin proxy respondents were used for the deceased cases and controls. The work history included questions on specific jobs, pesticides, and organic solvents. Exposures up to the year prior to diagnosis or corresponding reference year for controls were included in the analysis, which was conducted using conditional logistic regression. Increased risks were not seen with either chlorophenol exposure (OR 1.1, 95% CI 0.7, 1.8) or pentachlorophenol (OR 1.2, 95% CI 0.7, 1.8). A higher risk was seen with pentachlorophenol exposures that occurred between >20 and 30 years before diagnosis (OR 2.0, 95% CI 0.7, 5.3) compared with >10 – 20 years (OR 1.0, 95% CI 0.3, 2.9) or >30 years (OR 1.1, 95% CI 0.7, 1.8). The authors noted that chlorophenols had been banned from use in Sweden in 1977 (or, as noted in Hardell and Eriksson, 2003, in January 1978), resulting in a different exposure period relative to diagnosis for cases included in this study compared to their earlier study conducted among cases diagnosed between 1974 and 1978 (Hardell et al., 1994, 1981).

Hardell and Eriksson (2003) discuss the trends in use of phenoxyacetic acids and chlorophenols in relation to trends in the incidence of non-Hodgkin's lymphoma. Exposures to these compounds peaked in the 1970's; incidence rates increased from 1960 to the late 1980's and then were relatively steady through 2000. The authors note that these two trends are consistent with a relatively short latency period between first exposure and disease onset.

Two other case-control studies of non-Hodgkin's lymphoma assessed occupational exposure to chlorophenols with limited data specifically relating to potential exposure to jobs or activities with likely exposure to PCP (Woods et al., 1987; Pearce et al., 1986b) (Table 4-3). These studies reported no or weak (ORs <1.5) associations with chlorophenols, but somewhat stronger risks with some specific jobs involving wood preservation or fencing work. Smith and Christophers (1992) included Hodgkin's and non-Hodgkin's lymphoma in a small (52 cases) study conducted in Australia using the area cancer registry. One cancer control and one population-based control (from electoral rolls) were matched to each case based on age and place of residence. The measure of association (point estimate or statistical significance), based on the conditional logistic regression analysis of the matched triad data for PCP was not presented, but this type of exposure was noted in four cases, one population control and three of the cancer controls.

Case-control studies of soft tissue sarcoma. As with the studies of lymphoma, the case-control studies of soft tissue sarcoma can be categorized based on the level of detail of the PCP

assessment (Table 4-4). In the international nested case-control study by Kogevinas et al. (1995) described above, 12 cases of soft tissue sarcoma and 44 matched controls were identified among the 13,989 workers exposed to phenoxy herbicides or chlorophenols. None of these cases or controls had been exposed to PCP. A meta-analysis of four separate but related (in terms of exposure assessment methodology and other design features) case-control studies conducted in different areas of Sweden (Eriksson et al., 1990; Hardell and Eriksson, 1988; Hardell and Sandstrom, 1979; Eriksson et al., 1981) (Table 4-5) was published in 1995 (Hardell et al., 1995). The methodology was based on the process described above for a study of lymphoma by Hardell et al. (1994, 1981).

Table 4-5. Summary of case-control studies of chlorophenol and soft tissue cancer risk included in Hardell et al. (1995) meta-analysis

Reference	Region of Sweden	Case accrual	Age and sex criteria	n cases (percent deceased), n controls[a]
Hardell and Sandstrom (1979)	Umeå (northern)	1970–1977, hospital records	males, ages 26–80, controls matched by vital status, sex, age, and area	52 cases (60% deceased), 208 controls
Eriksson et al. (1981)	Five counties, (southern)	1974–1978, cancer registry	age and sex not specified, controls matched by vital status, age, and area	110 cases (35% deceased), 220 controls
Hardell and Eriksson (1988)	Three counties (northern)	1978–1983, cancer registry	males, ages 25–80, controls matched by vital status, age, and area	54 (67% deceased), 311 population controls (33% deceased), 179 cancer controls (59% deceased)
Eriksson et al. (1990)	Upsala (middle)	1978–1986, cancer registry	males, ages 25–80, controls matched by vital status, age, and area	218 (64% deceased), 212 controls

[a]The matching design used in all of the studies except Hardell and Eriksson (1988) resulted in an equal proportion of deceased cases and controls within each study.

Population controls were identified through a population registry or the national death registry, and were individually matched to the cases by age and area of residence. A total of 434 cases and 948 controls are included in the meta-analysis. Work history data was obtained through a self-administered questionnaire (completed by next-of-kin for deceased cases and controls) with follow-up phone interview (if needed to clarify responses). The work history data were used to create an exposure measure for specific chemicals, including various forms of phenoxyacetic acids and chlorophenols. Exposures in the 5 years immediately preceding diagnosis (or a corresponding reference year for controls) were excluded to account for a minimum latency period, and only "high" exposures (defined as 1 week or more continuously or at least 1 month in total) are included in the meta-analysis. A strong association was observed

between high exposure to PCP and soft tissue sarcoma risk (OR = 2.8, 95% CI 1.5–5.4). The primary strength of this meta-analysis is the relatively large number of cases obtained, which is difficult to achieve in single-site studies of this rare disease.

The studies used in the meta-analysis were conducted by the same group of investigators using a relatively common protocol across studies, which makes them suitable for this kind of combined analysis. The exposure assessment was relatively detailed. There was a relatively high proportion of deceased cases (and controls) in these studies (reflecting the high mortality rate in this disease). The completeness and level of detail of the work history and exposure data are likely to be lower in proxy- compared with self-respondents, resulting in a loss of precision and possibly attenuation to the null.

The other three case-control studies of soft tissue sarcoma risk with more limited data pertaining to PCP (Smith and Christophers, 1992; Woods et al., 1987; Smith et al., 1984) are summarized in Table 4-4. These studies present variable results pertaining to various jobs with potential exposure to PCP.

Case-control study of multiple myeloma. Pearce et al. (1986a) conducted a case-control study of farming-related exposures and multiple myeloma risk in New Zealand. Men less than age 70 years who had been hospitalized with a diagnosis of multiple myeloma (ICDs code 203) from 1977 to 1981 were recruited as cases. Controls, drawn from the Cancer Registry, were matched by age and sex (all men) to the cases. A structured interview, completed by 76 (82%) of the 93 eligible cases and 315 (81%) of the 389 eligible controls, was used to collect data pertaining to work history, with a particular focus on farming-related activities. There was little evidence of an association with the general category of chlorophenol exposure (OR = 1.1, 95% CI 0.4–2.7) and work in a sawmill or timber merchant (OR 1.1, 95% CI 0.5–2.3). Stronger associations were seen with a history of doing fencing work (OR 1.6, 95% CI 0.9–2.7) and jobs that involved potential exposure to chlorophenols in a sawmill or as a timber merchant (OR 1.4, 95% CI 0.5–3.9).

Case-control study of leukemia and brain cancer in children and young adults. Ali et al. (2004) recently reported results from a case-control study of leukemia (ICDs–9th revision codes 204–208) and brain cancer (benign and malignant, ICDs–9th revision codes 191, 192, 194.3, 194.4, and 225) in patients less than age 30 at diagnosis in Kaoshiung, Taiwan. Incident cases were drawn from a cancer registry and reviewed by a pathologist to confirm diagnoses. Population-based controls were drawn using a randomization scheme based on personal identification numbers, and were matched to the age and sex distribution of the cases. (The authors did not describe the matchings as to whether individual- or frequency-matching was used; unconditional logistic regression was used in the analysis and EPA assumes that frequency-matching was used.) The mean age of the brain cancer and leukemia cases were 18 and 11 years, respectively. Participation rates for controls were 61% for the brain cancer controls and 56% of the leukemia controls. Occupational history (name of company, location,

industry, duties, hours per week, and start and end dates) for jobs held more than 6 months since age 16 was obtained using a structured interview with each of the parents. Additional interviews were conducted with any patient (or control) who was at least 16 years old. The Taiwanese occupational and industrial coding system was used to assign 4-digit job codes based on this information. The specific time periods of exposure examined in the study were preconception (any job ending more than 1 year before the child's birth), prenatal (any job held between 1 year prior to the child's birth and the child's birth), and postnatal (a job held after the child's birth). Analyses were conducted using conditional logistic regression, adjusting for smoking history (of the participant and the parents) and exposure to medical radiation. Strong, but imprecise given the sample size, associations were seen between paternal work as a wood-treater and risk of leukemia (for any exposure period, five exposed cases, two exposed controls, OR = 16.0, 95% CI 1.8–145.4; for preconception period, four exposed cases, one exposed control, OR = 12.2, 95% CI 1.4–109.2; for perinatal period, four exposed cases, one exposed controls, OR 13.0, 95% CI 1.4–125.5). No other information is available pertaining to the specific material used by these workers (personal communication, email from Dr. David Christiani, Harvard School of Public Health, Boston, Massachusetts, to Dr. Glinda Cooper, U.S. EPA, dated 2006).

4.1.1.4. *General Issues—Interpretation of the Epidemiologic Studies*

The strongest of the cohort studies, in terms of design, is the large sawmill cohort study conducted in British Columbia, Canada and recently updated by Demers et al. (2006). As noted previously, important design features that add to the strengths of this study include its size (n = 23,829 workers), the exposure assessment procedure developed specifically to address the exposure situations and settings of the study, use of an internal referent group, analysis of PCP and TCP exposures, the low loss to follow-up, and the use of a population-based cancer registry that allowed for the analysis of cancer incidence. In contrast, the other cohort studies in a manufacturing plant (Ramlow et al., 1996) and a plywood mill (Robinson et al., 1987) were much smaller (n = 770 and 2,283, respectively), and did not present analyses that allow for differentiation of risk between potential co-exposures (e.g., dioxins and furans in the manufacturing plant, and formaldehyde in the plywood worker cohort). Even with the large size of the British Columbia sawmill cohort, however, there is limited statistical power to estimate precise associations with relatively rare cancers.

Case-control studies offer the potential for increased statistical power for assessing associations with rare cancers such as liver cancer and various forms of lymphomas; however, there is a considerable range in the detail and quality of the exposure assessment used in case-control studies. Population-based case-control studies rarely include specific exposure measurements taken at specific worksites of individual study participants. Although it is more difficult to determine absolute exposure levels without these individual measurements, the exposure assessment methodology does allow ranking of exposure levels and useful between-

group comparisons of risk. Among the case-control studies with data pertaining to cancer risk and PCP exposure, the studies with the strongest designs in terms of exposure assessment are the nested case-control study by Kogevinas et al. (1995), conducted within a large, multinational cohort of workers, and the collection of studies from Sweden (Hardell et al., 1995, 1994). These studies used population-based cancer registries for case ascertainment. The nested case-control study included detailed information pertaining to exposures for specific jobs, periods, and locations. The Swedish studies obtained detailed information about work histories (rather than just the usual or most recent job). The inclusion of work history from interviews with next-of-kin (for cases and controls) in the Swedish studies, however, is most likely to result in nondifferential misclassification of exposure, and thus attenuation in the observed associations.

Although there are demographic risk factors (e.g., age, sex, race) for non-Hodgkin's lymphoma, multiple myeloma, and soft tissue sarcoma, "lifestyle" behaviors (e.g., smoking history, alcohol use) have not been associated with these diseases. The large cohort study of sawmill workers by Demers et al. (2006), the smaller cohort study by Ramlow et al. (1996), and the nested case-control study by Kogevinas et al. (1995) all used internal comparison groups, which would also reduce the potential influence of confounders.

Contamination of PCP with dioxins and related by-products is known to occur as part of the production process. Several studies have examined the level of various dioxins and furans among workers in the PCP and trichlorophenol production workers at the Michigan Division of the Dow Chemical Company (Collins et al., 2007, 2006; Ott et al., 1993). The primary contaminants are hexa-, hepta-, and octa-chlorinated dibenzodioxins and higher-chlorinated dibenzofurans, rather than 2,3,7,8-TCDD.

There are several reasons that it is unlikely that the associations observed in the epidemiologic studies described above are due to these contaminants. Although 2,3,7,8-TCDD is associated with an increased risk of cancer, the available epidemiologic studies most consistently demonstrate this association with all cancers, rather than with individual cancers (NAS, 2006, Steenland et al., 2004). In contrast, none of the epidemiologic studies of PCP exposure have demonstrated an increased risk for all cancers, but there is evidence of associations (ORs, some of which are relatively strong) with various forms of lymphopoietic cancers (non-Hodgkin's lymphoma, multiple myeloma) and soft tissue sarcoma. Thus, the patterns observed differ substantially for PCP and dioxins.

Another argument against the influence of contaminants as the explanation for the observations pertaining to PCP is based on the comparisons within a study of effects of different chemicals. In the nested case-control study conducted within the large international cohort of workers exposed to phenoxy herbicides or chlorophenols (Kogevinas et al., 1995), the observed association between PCP exposure and non-Hodgkin's lymphoma (OR = 2.75, 95% CI 0.45–17.0) was stronger than the associations observed with the other dioxin and furan exposures, and there was little evidence of an association with other types of chlorophenols. Also, in the large

cohort study of sawmill workers by Demers et al. (2006), the associations with multiple myeloma were considerably stronger (based on RR), and the association with non-Hodgkin's lymphoma were similar or somewhat stronger, for PCP than for TCP, but there is little difference in the contaminants. The levels of contaminants are similar between the two chemicals, except that in PCP, the levels of octachlorodibenzo-p-dioxin (OCDD) and octachlorodibenzofuran are greater compared with those found in TCP (Schwetz et al., 1974a, b).

De Roos et al. (2005) recently reported results from a case-control study of non-Hodgkin's lymphoma that examined plasma levels of various polychlorinated biphenyls, dioxins, furans, and pesticides (PCP was not included in their analyses). There was no association between OCDD levels and lymphoma risk. The strongest association was seen with 1,2,3,4,7,8-hexachlorodibenzofurans, with an OR of 2.64 (95% CI 1.14–6.12) per 10 pg/g lipid. However, in a recent study of the Dow Chemical Company chlorophenol production workers in Michigan (Collins et al., 2007), the biggest difference in serum concentration of dioxin and furan congeners among PCP exposed workers compared with various referent groups was in OCDD levels (mean 2594, 509, and 439 pg/g lipid in the PCP workers, a worker non-exposed comparison group, and a community comparison group, respectively; $p < 0.05$ for comparisons between PCP and each of the referent groups). Much smaller elevations (i.e., mean values of approximately 10 pg/g lipid compared with 8 pg/g lipid) were seen for some of the hexa- or heptachlorodibenzofurans, but the authors noted there was little evidence of increased furan levels in the PCP exposed workers. Collins et al. (2007, 2006) also noted that although furan contaminants have been detected in commercial PCP, they have rarely been found in blood samples from PCP workers. Thus, it is unlikely that the observations pertaining to non-Hodgkin's lymphoma risk and PCP exposure can be attributed to heptachlorodibenzofuran. McLean et al. (2009a) also reported increased levels of OCDD in serum samples collected from PCP exposed sawmill workers 20 years after the last exposure to PCP, with mean levels of 309.25 and 157.83 pg/g lipid in exposed and non-exposed workers, respectively; data on furan levels were not provided.

The classifications used for the various subtypes of lymphomas, leukemias, and sarcomas can be confusing and may not be applied similarly in different studies, particularly when conducted over different time periods, or in different locations by different investigators. This potential inconsistency may contribute to differences in results for these subtypes seen across different studies, but any differences in disease definitions should not produce a biased result within a study since the disease classification methods in the available studies (e.g., Demers et al., 2006; Hardell et al., 1995) were independent of the exposure classification system.

4.1.1.5. *Specific Cancers*

Considering the issues described above with respect to the strengths and limitations of the available epidemiologic studies, the following summary of the evidence relating to PCP exposure and specific types of cancer can be made.

Liver cancer. An increased risk of liver cancer in relation to PCP, but not TCP exposure, was seen in the large cohort study of 23,829 sawmill workers in British Columbia (Demers et al., 2006). There was little evidence of an increased risk when considering a 10- or 20-year exposure lag period. The difference between the results in the no-lagged and lagged analyses may reflect the effect of PCP as a promoter, rather than an initiator of liver cancer; alternatively, it may reflect the influence of chance given the relatively low statistical power, and thus lack of precision, inherent in a study of this relatively rare cancer even in this large-sized cohort. No case-control studies of liver cancer risk in relation to PCP exposure were identified; the plywood mill workers cohort study (Robinson et al., 1987) focused on lymphatic and hematopoietic cancers and did not present liver cancer data; no cases of liver cancer were observed in the small cohort study of 770 men in a PCP manufacturing plant (Ramlow et al., 1996). The available epidemiologic studies, in combination with the observation of liver tumors in mice (NTP, 1989), suggest a relationship between PCP and carcinogenic effects, although it should be noted that this determination is based on limited human data.

Lymphomas (non-Hodgkin's lymphoma, multiple myeloma). There was substantial evidence of an association between PCP exposure and the incidence of non-Hodgkin's lymphoma and multiple myeloma, including an exposure-response trend across categories reflecting higher exposures, in the large cohort study of sawmill workers (Demers et al., 2006). For multiple myeloma, the risk ratios in the highest category of exposure were strong (>4.0), and there was no evidence of similar patterns in the analyses of TCP exposure. For non-Hodgkin's lymphoma, Demers et al. (2006) observed approximately a twofold increased risk in the highest two categories of exposure, with a slight attenuation seen in the mortality analysis. An attenuation of the exposure-response in the highest exposure category is commonly seen in epidemiologic studies of occupational cohorts (Stayner et al., 2003).

The nested case-control study by Kogevinas et al. (1995), conducted within the combined international cohorts of exposed phenoxy herbicide workers, also provides support for an association between PCP (but not other chlorophenols) and non-Hodgkin's lymphoma risk. One case-control study in Sweden with a relatively specific exposure measure of PCP also reported very strong associations (OR = 8.8) with non-Hodgkin's lymphoma. A subsequent study by the same investigators did not observe this type of association (OR 1.2). There are no case-control studies of multiple myeloma with a similarly focused type of exposure estimate. The available epidemiologic studies strongly suggest that PCP exposure is associated with non-Hodgkin's lymphoma and multiple myeloma risk. For the reasons described above, it is unlikely that this

association can be explained by co-exposures or contamination with other chlorophenols, dioxins, or furans.

Soft tissue sarcoma. There was no association between PCP exposure and increased risk of soft tissue sarcoma in the large sawmill worker cohort study by Demers et al. (2006). The trend, based on small numbers, was for a decreased risk with higher exposures. None of the 12 cases or 44 controls in the nested case-control study by Kogevinas et al. (1995) were exposed to PCP. However, the number of cases was insufficient to conclude that there is no association between exposure to PCP and soft tissue sarcoma. These observations, within both of these studies, reflect the difficulty in studying such a rare disease, even in large cohorts. In the collection of case-control studies conducted in Sweden, summarized by Hardell et al. (1995), a strong association (OR 2.8) was seen with their measure of PCP exposure (more than 1 week continuously or 1 month total), based on structured interviews. A limitation of these studies is the relatively large proportion of proxy respondents used (cases and matched controls), which is likely to result in a loss of precision and possible attenuation of the observed association. In almost all cases, the proportion of proxy respondents (i.e., because the case or control was deceased) was similar for cases and controls. The available epidemiologic studies provide some evidence of an association between PCP exposure and soft tissue sarcoma risk. The low incidence rate, combined with a need to consider histology to accurately make a classification, and a fairly high case fatality rate make it difficult to conduct definitive epidemiologic studies of this disease.

Childhood cancers. There was little evidence of an association between paternal exposure to PCP and the incidence of childhood cancers in the large sawmill worker cohort study (Heacock et al., 2000), although with only 40 incident cancers, even this large cohort is of limited statistical power for the analysis of these cancers. A small case-control study in Taiwan reported strong associations with childhood leukemia in relation to paternal exposure (particularly in the pre-conception and perinatal periods). The available epidemiologic data are too limited to assess with confidence whether parental, prenatal, or early childhood exposure to PCP affects risk of childhood cancers.

4.1.2. Studies of Noncancer Risk

4.1.2.1. *Case Reports of Acute, High-Dose Exposures*

One of the earliest reports recognizing the toxic effects of PCP in humans was published by Truhaut et al. (1952). The authors described the then current procedures for treatment of lumber to prevent rotting. Workers known as “treaters” soaked freshly sawn lumber in tubs containing a 3% solution of a mixture of 80% pentachlorophenate of sodium and 20% tetrachlorophenate of sodium. After soaking, the lumber was then carried to other workers called “stackers” to be put in stacks. Based on examinations of more than 100 lumber treaters, symptoms of PCP exposure included skin irritation with blisters, congestion of mucous

membranes of eyes and nose, loss of appetite, loss of weight, constriction of throat, respiratory stress, and fainting. Urine levels of PCP in 16 workers who had worked for 2 months as treaters were between 3 and 10 mg/L. Truhaut et al. (1952) also describe the deaths of two workers following exposure to PCP. Autopsy findings included liver poisoning, degenerative lesions in kidney, considerable edema in the lungs, the presence of PCP in liver, kidney, blood, stomach, intestine, heart, lung, and urine in one case, and considerable congestion and edema of the lungs and albumin in the urine in the other case.

An incident of accidental PCP poisoning occurred in a nursery for newborn infants in St. Louis in 1967 (Smith et al., 1996; Armstrong et al., 1969). Sodium pentachlorophenate had been used as an antimildew agent by the hospital laundry. Nine cases of illness were seen with fever and profuse sweating. As the disease progressed, respiratory rates increased and breathing became labored. Other common findings included rapid heart rate, enlarged liver, and irritability followed by lethargy. Laboratory tests showed progressive metabolic acidosis, proteinuria, increased levels of blood urea nitrogen, and x-rays suggestive of pneumonia or bronchiolitis. Two of the cases were fatal. The only source of exposure for the infants was skin absorption of the residues of sodium pentachlorophenate on the diapers, undershirts, and bedding. The product label warned against use in laundering diapers and the amount used was 3–4 times the amount recommended for regular laundry. Analysis of freshly laundered diapers showed a quantity of PCP ranging from 1.4 to 5.7 mg per diaper. One infant had 11.8 mg of PCP per 100 mL of serum before a transfusion was performed. A fatal case was found to have 2.1–3.4 mg per 100 grams in various body tissues. The average duration of the hospital stay in the nursery (when contaminated diapers were used) until the appearance of the first symptoms was 9 days.

Acute poisonings, including two fatalities, were reported in a study of workers in wood preservative manufacturing plants (Wood et al., 1983). A general air sample taken from the work area of one of the deceased workers found PCP levels of 4.6 mg/m^3, which is 9 times the Occupational Safety and Health Administration standard. Another case report described the occurrence of pancreatitis in a wood worker (joiner) who had been applying a wood preservative that contained PCP and zinc napththanate (Cooper and Macaulay, 1982). Gray et al. (1985) reported the case of a 33 year-old man who used a jackhammer to break up large blocks of PCP which were ground into powder. He developed lethargy, rapid respiration, and sweating, which led to his hospitalization, coma, pulmonary edema, and death.

From 1993 through 1996, 122 unintentional exposures were reported to the Toxic Exposure Surveillance System of the American Association of Poison Control Centers. Children under 6 years of age were involved in 32 of the exposures, and half of these were followed to determine outcome. Only five of the children were reported to have developed symptoms, all of which were minor. Six of the children were seen in a health care facility and one was hospitalized. There were 90 exposures in adults and older children, 30 of which had a minor outcome, nine with moderate outcome. One case was considered life-threatening. Thirty-four

cases were seen in a health care facility, two were hospitalized, and one was admitted for critical care.

Detailed descriptions of 71 cases of PCP exposure and health effects submitted to the California Pesticide Illness Surveillance Program (1982–1996) were evaluated. Irritative effects to the eye and skin were observed in 58% of the total reports of illness in California, while the remaining 42% exhibited effects systemic in nature, including symptoms of headache, nausea, and difficulty breathing. Only cases with a definite, probable, or possible relationship were reviewed. PCP was judged to be responsible for the health effects in 48 of these cases. Only half of the systemic cases were classified as having a probable or definite relationship between the exposure and the health effects. One individual was hospitalized in 1982 for skin grafts due to second and third degree burns after carrying PCP-treated lumber for 4 weeks. The burns were reported to the shoulder, neck, chin, back, and thigh, and were characterized as an allergic reaction by one investigator.

Dust and mist concentrations >1.0 mg/m^3 can result in painful irritation of the upper respiratory tract resulting in violent sneezing and coughing in persons not previously exposed to PCP (U.S. EPA, 1980). Some nose irritation has been reported at levels as low as 0.3 mg/m^3.

4.1.2.2. *Studies of Clinical Chemistries, Clinical Examinations, and Symptoms*

Chloracne has been often reported in studies of workers involved in the production of chlorophenols. Contamination with chlorinated dioxins and dibenzofurans is a likely cause of this association. Cole et al. (1986) describe a case of chloracne in a carpenter with substantial, prolonged dermal contact to PCP-treated lumber. Several studies have reported a high prevalence of chloracne among workers involved in the manufacture of PCP. Bond et al. (1989) examined 2,072 workers at the Dow Chemical Company manufacturing plant in Michigan. O'Malley et al. (1990) examined 648 workers in Illinois. Cheng et al. (1993) examined 109 workers at a production plant in China. The prevalence of chloracne was 15% in Michigan, 7% in Illinois, and 73% in China.

PCP was used extensively in Hawaii as a wood preservative for protection against termites and fungi endemic to the tropical climate. Studies of the health effects in workers occupationally exposed, and in the general population exposed through residential contact and diet, were begun in the 1960s (Bevenue, 1967). In a study of 18 exposed workers examined with serial blood and urine measures before and after a 21-day vacation, creatine clearance and phosphorus reabsorption were significantly decreased during the work period compared with the vacation period (Begley et al., 1977). Klemmer et al. (1980) reported data from a study of 47 Hawaiian workers involved with treatment of wood products with PCP, 333 workers with mixed exposures to various pesticides while working as farmers or pest control operators, and 42 controls with no history of occupational pesticide exposure (total n = 422). Blood and urinary measures of PCP were elevated in the exposed workers, particularly among those who had

worked with an open-vat process (e.g., mean serum concentrations 3.78, 1.72, 0.25, and 0.32 ppm in the open-vat wood treaters, pressure-tank wood treaters, farmers and pest control operators, and controls). Results of clinical laboratory analyses showed that PCP exposure was highly associated with increased numbers of immature leucocytes (band cells), increased levels of blood plasma cholinesterase, alkaline phosphatase (ALP), gamma-globulin, basophils, and uric acid, and reduced serum calcium. These analyses were limited to individuals with no missing data for any of the parameters, and included only 7 open-vat wood treaters, 10 pressure-tank wood treaters, 155 farmers, and pest control operators, and 17 controls. Age-standardized prevalence rates for conjunctivitis, chronic sinusitis, and chronic upper respiratory conditions were approximately 3 times higher among the workers exposed to PCP than among the controls. Prevalence rates of infections of the skin and subcutaneous tissue and of gout were approximately 1.7 times higher in the PCP-exposed individuals. The authors noted that the conjunctivitis cases only occurred among workers involved in pressure treatment and, therefore, had mixed exposure to PCP and other chemicals, and that the increased prevalence of gout may have been due to a greater proportion of Filipinos in the PCP-exposed group, since the prevalence of this condition is increased in this ethnic group.

Gilbert et al. (1990) examined clinical and laboratory parameters in another study of male wood treaters in Hawaii. The 88 study participants were drawn from a total of 182 workers who had worked for long periods and had chronic, low-level exposure to wood-treating chemicals including PCP. Exposed workers had to be currently employed in a Hawaiian wood treatment company for at least 3 months at the time of recruitment for the study or have been previously employed at least 12 months in a Hawaiian wood treatment company since 1960, including at least one 3-month period of continuous employment as a wood treater. A comparison group of 58 men was selected from various unions (e.g., carpenters, masons) and from friends and relatives of the exposed group. The comparison group was similar to the age, race, level of physical activity, and weight distribution of the exposed group. The level of urinary PCP was higher among the exposed (mean 174 and 35 ppb in the exposed and comparison groups, respectively). The clinical examination of study participants included a complete review of systems, lipid profile, and liver and kidney function tests. The authors reported no statistically significant differences between the groups in the elements of the clinical examination or symptoms (e.g., fever, skin rash, eye irritation, wheezing, cough). Although a few of the laboratory results (e.g., heart rate, systolic blood pressure) differed between cases and controls, additional analyses of trends across PCP exposure groups (based on urinary values) did not provide evidence of differences that could be attributed to this exposure.

Walls et al. (1998) examined medical history and current symptoms in 127 sawmill workers in New Zealand, many of whom were self-identified as having health concerns related to PCP exposure. Study participants were primarily recruited through the Wood Industries Union of Aoteoros and timber companies. Many also had exposures to other chemicals typically

used in the timber industry (e.g., arsenic) and to organopesticides. Data on occupational and lifestyle histories (e.g., tobacco and alcohol use), exposure to PCP, medical history, and current symptoms were collected using a structured questionnaire. An exposure metric incorporating length of PCP exposure and a cumulative score for types of PCP work, type of vehicle, use of personal protection, and intensity of exposure was calculated for each participant. Based on this exposure metric, participants were categorized into three groups: low (n = 45), medium (n = 39), and high (n = 43) exposure. There was no control group. An increased prevalence of weight loss, fevers, excess fatigue, upper respiratory tract symptoms, history of emphysema or bronchitis, and current or history of nausea was seen in the high-exposure group, and for many of these symptoms, an exposure-effect gradient was seen across the three exposure groups (trend $p \leq 0.05$). The authors describe these results as consistent with their clinical impressions, and as hypothesis generating observations that warrant additional research of a representative sample of workers exposed to PCP.

McLean et al. (2009b) followed up the findings of Walls et al. (1998) with an expanded study of former New Zealand sawmill and timber workers. Employment records from three employers (two sawmills and the New Zealand Forest Service) covering the period from 1970 to 1990 (McLean et al., 2007) were obtained and used to identify a cohort of workers. This follow-up study included workers who had worked for more than 12 months and who were alive after 2003 and living in New Zealand. After excluding 249 individuals who declined to participate (n = 146) or who were not able to be contacted by post (n = 103), a pool of 776 potential participants remained. From this pool, 338 were recruited and consented to participate, and 293 completed the study. The study involved an in-person interview, clinical examination (including a standardized neurological exam), and a blood sample (used for a non-fasting blood glucose test). These activities were conducted either at a medical center or in a participant's home; the exam was conducted separately from the interview, by a different member of the study team. The interview included more detailed information about work history and tasks and a health history. This history included questions about diagnosis with respiratory and other conditions and 10 physical symptoms (Table 4-6).

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Table 4-6. Prevalence of medical conditions and physical symptoms, and associations with PCP exposure, in 293 timber workers in New Zealand

History of:	Non-exposed (n=177)		Exposed (n=116)				Low Exposure[a] (n=58)				High Exposure[a] (n=58)			
	n	(%)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)	n	(%)	OR	(95% CI)
Conditions														
Asthma	30	(17.1)	27	(23.3)	1.46	(0.79, 2.68)	11	(19.0)	1.56	(0.53, 2.53)	16	(27.6)	1.79	(0.87, 3.70)
Nasal allergies	75	(42.6)	37	(31.9)	0.62	(0.37, 1.03)	18	(31.0)	0.61	(0.32, 1.16)	19	(32.8)	0.59	(0.31, 1.11)
Eczema	49	(27.8)	44	(37.9)	1.50	(0.90, 2.50)	19	(23.8)	1.20	(0.62, 2.29)	25	(43.1)	1.87	(0.99, 3.51)
Acne	61	(34.7)	35	(30.4)	0.87	(0.52, 1.47)	18	(31.0)	0.88	(0.46, 1.69)	17	(29.8)	0.86	(0.44, 1.68)
Chronic bronchitis	22	(12.5)	15	(13.0)	1.01	(0.48, 2.13)	5	(8.6)	0.65	(0.23, 1.85)	10	(17.5)	1.43	(0.60, 3.38)
Tuberculosis, pleurisy or pneumonia	13	(7.4)	24	(20.7)	3.04	(1.46, 6.33)	13	(22.4)	3.41	(1.46, 7.94)	11	(19.0)	2.68	(1.11, 6.48)
Diabetes	8	(4.6)	10	(8.6)	1.95	(0.73, 5.23)	7	(12.1)	2.93	(0.99, 8.72)	3	(5.2)	1.07	(0.27, 4.31)
Thyroid disorder	7	(4.0)	6	(5.2)	1.50	(0.47, 4.85)	2	(3.5)	1.00	(0.19, 5.11)	4	(6.9)	2.03	(0.54, 7.64)
Impaired kidney function	21	(11.9)	14	(12.2)	0.94	(0.43, 2.02)	6	(10.5)	0.83	(0.30, 2.26)	8	(13.8)	1.05	(0.41, 2.68)
Impaired liver function	15	(8.5)	18	(15.5)	1.94	(0.92, 4.10)	11	(19.0)	2.42	(1.03, 5.72)	7	(12.1)	1.46	(0.55, 3.88)
Physical symptoms														
Unintentional weight loss	12	(6.8)	14	(12.1)	1.57	(0.68, 3.62)	9	(15.5)	2.13	(0.83, 5.47)	5	(8.6)	1.05	(0.34, 3.22)
Unexplained persistent fevers	7	(4.0)	10	(8.6)	2.08	(0.76, 5.73)	8	(10.3)	2.58	(0.82, 8.09)	4	(6.9)	1.60	(0.44, 5.79)
Persistent fatigue	37	(21.0)	31	(26.7)	1.26	(0.72, 2.22)	16	(27.6)	1.31	(0.66, 2.63)	15	(25.9)	1.21	(0.59, 2.46)
Eye discomfort (reddened and dry)	49	(27.8)	28	(24.1)	0.88	(0.51, 1.53)	14	(24.1)	0.87	(0.43, 1.74)	14	(24.1)	0.89	(0.43, 1.81)
Pins and needles, hands or feet	82	(46.6)	52	(44.8)	0.80	(0.48, 1.31)	23	(39.7)	0.68	(0.36, 1.28)	29	(50.0)	0.93	(0.50, 1.74)
Numbness, hands or feet	58	(33.0)	38	(32.8)	0.95	(0.57, 1.60)	14	(24.1)	0.65	(0.32, 1.29)	24	(41.4)	1.34	(0.71, 2.51)
Loss of muscle power, hands or feet	25	(14.2)	21	(18.0)	1.64	(0.69, 2.58)	10	(17.2)	1.31	(0.58, 2.98)	11	(19.0)	1.37	(0.61, 3.05)
Recurrent nausea	6	(3.4)	12	(10.3)	2.42	(0.85, 6.87)	3	(5.2)	1.18	(0.28, 5.08)	9	(15.5)	3.71	(1.21, 11.4)
Recurrent diarrhea	8	(4.6)	14	(12.1)	2.68	(1.07, 6.71)	10	(17.2)	4.08	(1.51, 11.0)	4	(6.9)	1.42	(0.40, 4.98)
Recurrent bowel upsets	18	(10.2)	15	(12.9)	1.28	(0.61, 2.72)	10	(17.2)	1.81	(0.78, 4.28)	5	(15.2)	0.80	(0.28, 2.29)

[a] Cumulative exposure metric, based on product of intensity and duration; low score = 0 -120; high score = ≥120. Similar patterns seen with the intensity score.

Source: McLean et al. (2009b, 2007)

Exposure status was based on review of job history records, with a semi-quantitative intensity score based on job title (taking into account degree of direct contact with PCP) and specific high-exposure tasks (mixing PCP solutions, cleaning sludge from PCP dip tanks, and backpack spraying) (McLean et al., 2009b). A cumulative exposure measure was based on the product of the intensity and work duration data. Exposure classification was conducted separate from the clinical examination and interview. Among the 293 study participants, 177 and 116 were classified as non-exposed and exposed to PCP, respectively. Categories used for analysis of total intensity score were 2.0 to 4.9 (n = 86) and ≥5.0 (n = 30); categories for the cumulative exposure analysis were <120 (n = 58) and ≥120 (n = 58).

Analyses were adjusted for age (as a continuous variable, gender, and smoking status (never, former, and current). Comparing the non-exposed and exposed categories, an association was seen between exposure and chronic respiratory disease (OR 3.04, 95% CI 1.46, 6.33) and recurrent diarrhea (OR 2.68, 95% CI 1.07, 6.71). Other outcomes that were elevated (OR ≥ 1.5), with higher risks (OR approximately 2.0 or higher) seen in the higher exposure groups, were eczema, thyroid disorder, unexplained persistent fevers, and recurrent nausea (Table 4-6).

Two reports have described health effects of nonoccupational exposure to PCP (Lambert, 1986; CDC, 1980). The U.S. EPA conducted a survey of PCP-treated log homes and their occupants at the request of the Kentucky Department of Health Services (CDC, 1980). Environmental and medical data were collected for 32 individuals in 21 homes. No significant associations were reported between serum or urinary levels of PCP and health complaints, laboratory parameters of liver function, microsomal enzyme induction, renal function, neurological examination, or presence of lymphadenopathy. However, there was an association between a finding of skin abnormalities and serum and urinary levels of PCP. The types of skin abnormalities were not described. The author noted that skin abnormalities might lead to increased absorption of PCP resulting in higher biologic PCP concentrations in blood and urine, rather than PCP being a cause of skin abnormalities. In another report of nonoccupational PCP exposure, Lambert et al. (1986) describe the development of pemphigus vulgaris, a serious autoimmune disease involving successive blisters (bullae) in a 41-year-old man who had purchased a PCP-treated bookcase and in a 28-year-old woman who had several rafters in the living room treated with PCP. A third case involving urticaria (hives) occurred in a 35-year-old male who worked with PCP-treated wooden framework. The authors noted a “striking parallelism” in all three cases between the disease course and PCP serum levels and stated that these cases suggest “possible new hazardous effects of PCP.”

4.1.2.3. *Studies of Neurological Outcomes*

Two of the studies of general health effects described in this section also contain data pertaining to neurobehavioral function (Walls et al., 1998; Cheng et al., 1993). In the study of 127 sawmill workers in New Zealand by Walls et al. (1998), a questionnaire developed to screen

for neuropsychological impairment within the context of solvent exposures was used. This measure of neuropsychological dysfunction was associated with PCP exposure level, with 62% of the low-exposure group, 74% of the medium-exposure group, and 81% of the high-exposure group characterized as positive on this screening test (trend $p \leq 0.05$). Cheng et al. (1993) included a nerve conduction test in a study of workers at a PCP production plant and a comparison group of desalination plant workers. A slower conduction time was seen among workers (n = 10) in the trichlorobenzene building (in which non-γ-hexachlorocyclohexane was heated and decomposed into trichlorobenzene and hydrogen chloride) compared with the controls. However, there was no reduction in conduction time among workers in the other production areas.

Triebig et al. (1987) conducted a longitudinal study of nerve conduction velocity on 10 individuals who had worked with PCP or PCP-containing substances including TCP, γ-hexachlorocyclohexane (lindane), and aldrin for an average of 16 years (range = 4–24 years). Nerve conduction velocity measurements were available for comparison for years 1980 and 1984 for the 10 subjects. In addition, serum and urine concentrations of PCP were measured. Limited industrial hygiene data showed that PCP concentrations in the air during the subjects' employment were below the maximum allowable concentration of 500 μg/m^3. Results of biological monitoring showed serum concentrations of PCP between 38 and 1,270 μg/L (upper normal limit = 150 μg/L) and urine concentrations between 8 and 1,224 μg/L (upper normal limit = 60 μg/L) showing definite internal exposure. However, no significant changes in nerve conduction velocity during the period 1980–1984 were demonstrated in any of the subjects, and there was no observed correlation between nerve velocity and level of PCP exposure.

Peper et al. (1999) examined neurobehavioral measures in 15 women exposed to wood preserving chemicals in their residence and a comparison group of 15 unexposed women. Both groups were drawn from a larger study of women seen at a university hospital in Heidelberg, Germany, for reproductive and menopausal-related (but not neurological) complaints. Wood preserving chemicals, usually containing PCP and/or lindane, had been used on interior wood in this region. Exposure status was based on answers to a questionnaire pertaining to environmental risk factors (e.g., treatment of wood in the home) and serum levels of PCP and lindane. The exposed group consisted of women who indicated exposure to wood preserving chemicals for >5 years who had a blood level >25 μg/L PCP and 0.1 μg/L lindane. The mean (standard deviation) blood levels in the exposed and control groups, respectively, were 43.6 (31.2) μg/L and 11.8 (4.5) μg/L for PCP ($p = 0.001$), 0.085 (0.086) μg/L and 0.043 (0.025) for lindane ($p = 0.007$), and 0.497 (0.964 μg/L) and 0.268 (0.164 μg/L) for β-hexachloro-cyclohexane ($p > 0.05$). Neurobehavioral assessment included a 27-item questionnaire used to derive scores for three factors relating to attention (distractibility and slowing of mental processes, fatigue and slowing of practical activities, and motivation and drive), an emotional mood scale, the Beck Depression Inventory, and the Freiburg Personality Inventory to assess

primary personality traits. Study participants also underwent a neuropsychological examination focusing on tests sensitive to cortico-striatal dysfunction, an intelligence quotient (IQ) test, tests of attention and of psychomotor speed, visual and verbal span subtests of the Wechsler Memory Scale-Revised, and the "Tower of Hanoi task" test of motor skills. A close relative of each study participant also completed a rating scale of behavior. Several differences between the exposed and control groups in these neurological tests were seen, including higher (i.e., worse functioning) scores on the Beck Depression Inventory, three of the four measures of mood (depression, fatigue, irritability), and some of the memory and attention tests. These differences were all statistically significant ($p < 0.05$ with Bonferoni correction), although group means did not fall within a range that would be classified as "impaired". This set of analyses did not distinguish between the effects of PCP, γ-hexachlorocyclohexane, or other compounds, but serological measures of these exposures (PCP, γ-hexachlorocyclohexane, and β-hexachloro-cyclohexane) were used in analyses of the correlation between specific exposures and the neurological measures. Serum PCP level was inversely correlated ($r \sim -0.65$) with reading speed and naming speed, and positively associated ($r \sim 0.60$) with error rates in the paired-association test and the Benton visual retention test. These correlations were statistically significant adjusting for age, and were stronger than those seen with γ-hexachlorocyclohexane. In contrast, the correlations seen with γ-hexachlorocyclohexane were with measures of memory performance. Exposure to β-hexachlorocyclohexane was not correlated with any of the effect measures, and none of the exposures were correlated with the self-reported symptom data. This small study provides data suggesting the types of neurobehavioral effects that may be seen in chronic exposure to PCP.

The study of 293 sawmill and timber workers in New Zealand by McLean et al. (2009b), described in Section 4.1.2.2, also included 17 neuropsychological symptoms in the interview (Table 4-7), and a standardized neurological examination (Table 4-8). Adjusting for age (as a continuous variable), gender, and smoking status (never, former, and current), heart palpitations (OR 1.92, 95% CI 1.06, 3.50) and unexplained sweating (OR 2.10, 95% CI 1.14, 3.87) were associated with PCP exposure; for heart palpitations, a stronger risk was seen in the higher exposure group (Table 4-7). An association was also seen between exposure and straight leg raising (OR 2.10, 95% CI 1.16, 3.81), with a weaker association seen in the cranial nerve exam (OR 1.64, 95% CI 0.94, 2.88) (Table 4-8).

Table 4-7. Prevalence of neuropsychological symptoms, and associations with PCP exposure, in 293 timber workers in New Zealand

	Non-exposed (n=177)		Exposed (n=116)				Low Exposure[a] (n=58)				High Exposure[a] (n=58)			
Symptoms	**n**	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**
Short memory	72	(40.9)	47	(40.5)	1.02	(0.62, 1.68)	33	(38.4)	0.97	(0.56, 1.69)	14	(46.7)	1.17	(0.52, 2.64)
Often need to take notes	98	(55.7)	49	(42.2)	0.60	(0.37, 0.97)	35	(40.7)	0.58	(0.34, 0.99)	14	(46.7)	0.65	(0.29, 1.45)
Often go back to check things	82	(46.6)	57	(49.1)	1.16	(0.72, 1.89)	41	(47.7)	1.15	(0.67, 1.95)	16	(53.3)	1.22	(0.55, 2.71)
Hard to get meaning from reading	40	(22.7)	24	(20.7)	0.73	(0.40, 1.32)	18	(20.9)	0.76	(0.40, 1.45)	6	(20.0)	0.65	(0.24, 1.76)
Problem concentrating	55	(31.3)	38	(32.8)	0.97	(0.58, 1.64)	27	(31.4)	0.94	(0.53, 1.67)	11	(36.7)	1.06	(0.46, 2.44)
Feel depressed	32	(18.2)	30	(25.9)	1.57	(0.88, 2.82)	22	(25.6)	1.58	(0.84, 2.97)	8	(26.7)	1.55	(1.62, 3.90)
Abnormally tired	45	(25.6)	34	(29.3)	1.24	(0.72, 2.13)	26	(30.2)	1.30	(0.73, 2.33)	8	(26.7)	1.07	(0.44, 2.63)
Less interested in sex	28	(15.9)	24	(20.7)	1.40	(0.75, 2.63)	16	(18.6)	1.26	(0.63, 2.53)	8	(26.7)	1.85	(0.72, 4.74)
Heart palpitations	29	(16.5)	31	(26.7)	1.92	(1.06, 3.50)	20	(23.3)	1.65	(0.85, 3.19)	11	(36.7)	2.84	(1.18, 6.80)
Feel an oppression in chest	36	(20.5)	29	(25.0)	1.26	(0.71, 2.25)	18	(20.9)	1.02	(0.53, 1.97)	11	(36.7)	2.12	(0.90, 4.99)
Sweat with no reason	26	(14.8)	31	(26.7)	2.10	(1.14, 3.87)	23	(26.7)	2.15	(1.12, 4.16)	8	(26.7)	1.94	(0.75, 4.99)
Headache at least once a week	39	(22.2)	24	(20.7)	0.86	(0.47, 1.56)	18	(20.9)	0.90	(0.47, 1.72)	6	(20.0)	0.75	(0.28, 2.03)
Painful tingling	39	(22.2)	34	(29.3)	1.31	(0.75, 2.28)	25	(29.1)	1.37	(0.75, 2.50)	9	(30.0)	1.15	(0.48, 2.7)
Problem buttoning or unbuttoning	16	(9.1)	11	(9.5)	1.05	(045, 2.43)	8	(9.3)	1.07	(0.43, 2.69)	3	(10.0)	0.99	(0.26, 3.79)
Trouble sleeping	54	(30.7)	42	(36.2)	1.28	(0.77, 2.14)	31	(36.1)	1.30	(0.74, 2.26)	11	(36.7)	1.24	(0.54, 2.85)
Frequent mood changes	37	(21.0)	35	(30.2)	1.52	(0.86, 2.69)	26	(30.2)	1.64	(0.88, 3.04)	9	(30.0)	1.25	(0.50, 3.08)
Bothered by noise more than in past	72	(40.9)	52	(44.8)	1.11	(0.68, 1.81)	41	(47.7)	1.29	(0.76, 2.20)	11	(36.7)	0.71	(0.31,1.62)

[a] Cumulative exposure metric, based on product of intensity and duration; low score = 0 -120; high score = ≥120. Similar patterns seen with the intensity score.

Source: McLean et al. (2009b, 2007)

Table 4-8. Prevalence of abnormalities seen in neurological examination, and associations with PCP exposure, in 293 timber workers in New Zealand

	Non-exposed (n=177)		Exposed (n=116)				Low Exposure[a] (n=58)				High Exposure[a] (n=58)			
Test	**n**	**(%)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**	**n**	**(%)**	**OR**	**(95% CI)**
Cranial nerves	46	(26.4)	39	(33.9)	1.64	(0.94, 2.88)	27	(31.8)	1.45	(0.78, 2.68)	12	(40.0)	2.35	(0.97, 5.68)
Sensory exam by cotton wool	19	(10.9)	11	(9.5)	0.79	(0.35, 1.79)	7	(8.1)	0.71	(0.28, 1.83)	4	(13.3)	0.97	(0.29, 3.22)
Sensory exam by pin prick	20	(11.6)	11	(9.7)	0.75	(0.33, 1.68)	7	(8.4)	0.68	(0.27, 1.72)	4	(13.3)	0.92	(0.28, 3.04)
Vibration sense	13	(7.5)	6	(5.2)	0.68	(0.24, 1.94)	4	(4.7)	0.61	(0.18, 2.00)	2	(6.7)	0.93	(0.18, 4.78)
Joint position	4	(2.3)	3	(2.6)	1.21	(0.25, 5.78)	2	(2.4)	1.10	(0.19, 6.26)	1	(3.3)	1.78	(0.18, 17.3)
Two point discrimination	50	(28.7)	36	(31.3)	1.11	(0.65, 1.91)	29	(34.1)	1.26	(0.71, 2.25)	7	(23.3)	0.74	(0.28, 1.90)
Wasting	4	(2.3)	2	(1.8)	0.63	(0.10, 3.83)	0	(0.0)	--	--	2	(6.9)	2.74	(0.38, 19.7)
Power upper limb	5	(2.9)	1	(0.9)	0.33	(0.04, 3.05)	1	(1.2)	0.50	(0.05, 4.61)	0	(0.0)	--	--
Power lower limb	7	(4.1)	1	(1.0)	0.22	(0.03, 1.91)	0	(0.0)	--	--	1	(3.3)	0.82	(0.09, 7.68)
Reflexes	35	(20.1)	16	(13.9)	0.60	(0.31, 1.17)	13	(15.3)	0.69	(0.34, 1.41)	3	(10.0)	0.38	(0.11, 1.36)
Straight leg raising	28	(17.3)	32	(31.7)	2.10	(1.16, 3.81)	21	(28.4)	1.78	(0.92, 3.44)	11	(40.7)	3.28	(1.32, 8.11)
Gait	4	(2.5)	2	(1.8)	1.04	(0.17, 6.57)	1	(1.2)	0.52	(0.06, 4.81)	1	(3.3)	1.64	(0.17, 15.8)
Tests of coordination	8	(4.6)	3	(2.6)	0.64	(0.15, 2.59)	1	(1.2)	0.29	(0.03, 2.51)	2	(6.7)	1.64	(0.30, 8.99)

[a] Cumulative exposure metric, based on product of intensity and duration; low score = 0 -120; high score = ≥120. Similar patterns seen with the intensity score.

Source: McLean et al. (2009b, 2007)

4.1.2.4. *Studies of Reproductive Outcomes*

Two studies examined reproductive outcomes in relation to exposure to PCP and/or lindane in residences or places of work in Germany (Gerhard et al., 1999; Karmaus and Wolf, 1995). Karmaus and Wolf (1995) studied reproductive outcomes among daycare center workers who were exposed at their place of work to wood preservatives. Because of concerns about indoor air exposure to these chemicals, measurements of PCP concentrations in all daycare centers in Hamburg were conducted by the government in 1986. In 24 centers, PCP concentrations in the wood of more than 100 ppm were found. Indoor air concentrations of PCP, lindane, pentachlorodibenzo-dioxin, and pentachlorodibenzofuran were conducted in these centers. The median concentrations in these samples were 0.25 μg/m^3 for PCP, 0.2 μg/m^3 for lindane, and 0.5 pg/m^3 toxic equivalent factors for polychlorinated dibenzo-p-dioxins/ dibenzofurans. Women who worked in any of these daycare centers during a pregnancy and a comparison group of women who had worked in other daycare centers were recruited through the employer's insurance program. The study included 214 exposed women and 184 control women, with 49 pregnancies (32 live births) during an exposure period and 506 nonexposed pregnancies (386 live births). The nonexposed pregnancies included pregnancies among exposed women that did not occur while working at the place of exposure, and pregnancies among the controls. Study participants completed an interview focusing on occupational, lifestyle, and reproductive histories. Information on pregnancy outcomes, birth weight, and birth length was validated by review of medical cards for a subgroup of 220 (59%) participants. In analyses excluding twins and adjusting for age at conception and gestational age, employment at the high-exposure daycare centers during pregnancy was associated with an approximately 220 g decrease in birth weight and a 1.1 cm decrease in birth length.

Gerhard et al. (1999) conducted a study of 171 women who were referred to a gynecological clinic in Germany because of infertility or other gynecological and/or endocrine-related conditions to investigate possible effects of PCP exposure on the endocrine system. Exposure status was based on serum levels of PCP, with the "exposed" defined as ≥20 μg/L (n = 65). The other 106 women who served as controls (PCP levels <20 μg/L) were matched to the exposed women on age, underlying condition, and geographical region. Gonadotropin and estradiol analyses were based on blood samples taken on days 2–5 of the menstrual cycle, and progesterone was based on two samples taken during the luteal phase of the cycle. Thyroid stimulating hormone was measured in an unstimulated (baseline) sample and 30 minutes after administration of 200 μg of thyrotropin releasing hormone. Cortisol and various androgen hormones were also measured with a baseline sample and after administration of 0.25 μg of adrenocorticotrophic hormone.

The median PCP level in the PCP group was 35.9 μg/L compared to 9.5 μg/L for the controls. Small differences in follicle stimulating hormone (FSH) levels (median 5.9 and 6.9 mE/mL in exposed and controls, respectively, $p = 0.0053$) and triiodothyronine (T_3) (median

0.98 and 1.02 ng/mL in exposed and controls, respectively, $p = 0.046$) were observed. Euthyroid goiters were found more frequently in the PCP group than the controls (50 versus 30%). There was no difference in the baseline cortisol levels between the PCP and control groups, but a larger increase was seen in the PCP group after adrenocorticotrophic hormone stimulation. Baseline levels of testosterone and other androgens, and 17-hydroxypregnenolone, and 17-hydroxy progesterone were lower in the PCP group, but there was no difference between the PCP and control group in these hormone levels seen in response to the adrenocorticotrophic hormone stimulation. This study showed that relatively high serum PCP levels in women are associated with a number of endocrine effects, particularly related to androgen responsiveness, among patients seen for infertility and endocrine disorders.

Dimich-Ward et al. (1996) conducted a nested case-control study of reproductive outcomes among offspring of 9,512 male production and maintenance workers in the British Columbia sawmill workers cohort described in Section 4.1.1, Studies of Cancer Risk). Chlorophenates (primarily PCP and TCP) were used at the 11 sawmills in this study from 1950 to 1989, with TCP use increasing around the mid 1960s. These workers were the basis for the large cohort study reported by Demers et al. (2006) of cancer risks described in Section 4.1.1.2. (Studies of Cancer Risk—Cohort Studies). Marriage and birth records were linked to identify 19,675 children born to these fathers between 1952 and 1988, and born after their father began employment at the study sawmills. Cases of congenital anomalies were identified within these children through the linking of these birth records to the British Columbia Health Surveillance Registry. These outcomes were coded based on 3-digit ICD–9th revision categories. Other reproductive outcomes selected for study were prematurity (born at <37 weeks gestation), low birth weight (<2,500 g), small for gestational age (less than the 10th percentile of gestation-specific weight based on British Columbia births), neonatal deaths (death of a liveborn infant before age of 1 year), and stillbirths (pregnancy of at least 28 weeks gestation). For each case of any of these outcomes, five controls were chosen matching to the year of birth of the cases. Gender was an additional matching criterion for the congenital anomalies, and was used as an adjustment variable for the other outcomes. Exposure assessments for each job title were made by experienced workers for each mill for time periods characterized as having relatively constant exposure. Each worker's exposure estimate was calculated by multiplying this exposure constant by duration of employment in each job for each time period. The exposure measures used in the analyses included a cumulative exposure estimate for each of three time windows relative to time of conception (up to 3 months prior to conception, in the 3 months prior to conception, through the period of pregnancy), and a measure of the maximum exposure (hours/year) for any sawmill job up to 3 months prior to conception.

There was no association between any of the exposure measures and the risk of premature birth, low birth weight, small for gestational age, neonatal death, or stillbirth. Congenital anomalies of the eye (ICD–9th revision code 743, 22 cases) were associated with the

cumulative exposure measure for each of the three time periods (but most strongly for the measures limited to the 3 months prior to conception and to the pregnancy period). This was seen when analyzed as a continuous variable per 100 hours of estimated exposure (ORs 2.01 and 1.21 for the 3 months prior to conception and to the pregnancy period measures, respectively, $p < 0.005$) and in analyses comparing the 75th percentile with the 25th percentile of exposure (ORs 2.87 and 2.59 for the 3 months prior to conception and to the pregnancy period measures, respectively). Further analyses indicated that strong associations were seen with congenital cataracts (ICD–9th revision code 743.3, 11 cases). In the comparison of the 75th percentile with the 25th percentile of exposure, the ORs for this outcome were 5.68 and 4.34 for the 3 months prior to conception and to the pregnancy period measures, respectively. Weaker associations (ORs around 1.3 in the analyses by percentile) were seen for spina bifida (ICD–9th revision code 741, 18 cases) and for anomalies of genital organs (ICD–9th revision code 752, 105 cases). The strengths of this study include its large size and the specificity of the measured outcomes.

4.1.2.5. *Summary of Studies of Noncancer Risk*

Instances of PCP poisoning have been documented, indicating the potentially severe consequences of acute, high-dose exposures. Few studies have examined the effects of the lower exposures that occurred in occupational settings or through residential or environmental sources. Many of the available studies are relatively small (<50 participants) (Peper et al., 1999; Triebig et al., 1987; Klemmer et al., 1980; Begley et al., 1977) or may not be representative of the exposed population (Gerhard et al., 1999; Walls et al., 1998). Despite these limitations, there are indications of specific types of neurobehavioral effects seen with chronic exposure to PCP in non-occupational settings (Peper et al., 1999). A larger study of 293 former sawmill workers in New Zealand also suggests neuropsychological effects and respiratory diseases (McLean et al., 2009b). In addition, the large nested cohort study of reproductive outcomes in offspring of sawmill workers (Dimich-Ward et al., 1996) indicates that specific types of birth defects warrant additional research.

4.2. SHORT-TERM, SUBCHRONIC, AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

This section presents the available PCP toxicity studies that characterize the effects associated with PCP exposure to animals via the oral and inhalation routes. Although studies have been summarized and presented according to their route and duration of exposure, some of the toxicity studies within the database have utilized various forms of PCP. During manufacture of PCP, the chemical becomes contaminated with impurities. These impurities are other chlorophenols, such as TCP, chlorinated dibenzo-p-dioxins, and chlorinated dibenzofurans. Studies investigating the toxicity of PCP generally employ the technical grade, which is composed of approximately 90% PCP and 10% of the various contaminants. The tPCP is frequently found under the trade names Dowicide 7, Dowicide EC-7 (EC-7), Dow PCP DP-2

Antimicrobial (DP-2), Duratox, Fungol, Penta-Kil, and Permacide. Use of EC-7 and DP-2 are identified where possible; all other forms of technical grade PCP will be referred to in the document as tPCP. To achieve an analytical grade of PCP, an additional purification step to remove the contaminants that were simultaneously created during the manufacturing of PCP is required. Although the use of the analytical grade or aPCP is limited, there are several studies within the database that employ the relatively pure form of the chemical (99% purity). Where possible, the type of PCP utilized within the studies has been identified.

4.2.1. Oral Studies

4.2.1.1. *Short-term Studies*

Kerkvliet et al. (1982a) found that B6 mice treated with 1,000 ppm aPCP (average dose estimated as 195 mg/kg-day) for 4 days exhibited no changes in body weight compared with controls. Relative liver and spleen weights were significantly elevated 76 and 26%, respectively, compared with controls.

NTP (1999) reported a 28-day toxicity study in groups of 10 male and 10 female F344N rats administered aPCP (99% purity) in the diet at concentrations of 200, 400, 800, 1,600, or 3,200 ppm (average doses are estimated as 20, 40, 75, 150, and 270 mg/kg-day, respectively). One male and two females receiving 270 mg/kg-day died before the end of the study. Statistically significant decreases in the final mean body weights of males and female rats were observed at the two highest doses. Male body weights were reduced 14 and 47% at 150 and 270 mg/kg-day, respectively. Females exhibited 19 and 43% reductions in mean final body weights at the 150 and 270 mg/kg-day concentrations, respectively. Decreased food consumption was measured in male and females in the 150 and 270 mg/kg-day dose groups on day 1 and in males in the 270 mg/kg-day dose group on day 28. It is possible that the reduction in food consumption contributed to the decreased body weight at the two highest doses for both sexes. Microscopic effects of aPCP administration were confined to the liver (hepatocyte degeneration and centrilobular hypertrophy) and testes (degeneration of the germinal epithelium). The incidence and severity of hepatocyte degeneration were statistically, significantly increased in males receiving ≥40 mg/kg-day and in females receiving ≥75 mg/kg-day. The incidence of centrilobular hypertrophy was significantly increased only at 270 mg/kg-day in both sexes. Degeneration of the testicular germinal epithelium occurred in all males receiving 270 mg/kg-day but in none of the control or lower dose group males. Mild to chronic active inflammation was observed in the nasal sections of all control males and in some males of each dose group. NTP (1999) did not determine no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) values. The EPA determined that, for male rats, the NOAEL was 20 mg/kg-day and the LOAEL was 40 mg/kg-day, based on significant hepatocyte degeneration. In females, the NOAEL was 40 mg/kg-day and the LOAEL was 75 mg/kg-day, based on significant hepatocyte degeneration.

In an NTP (1989) study, groups of male and female B6C3F_1 mice were fed tPCP (90.4% purity), Dowicide EC-7 (91% purity), or aPCP (98.6% purity) for 30 days. There were 19 and 11 controls for the males and female groups, respectively; 15 mice/group treated with tPCP and 5 mice/group treated with EC-7 or aPCP. The administered doses corresponding to the dietary concentrations of 20, 100, 500, 2,500, or 12,500 ppm PCP are estimated as 4, 19, 95, 593, or 5,367 mg/kg-day for males and 5, 25, 126, 645, or 3,852 for females, respectively. Treatment-related effects included clinical signs, increased mortality, decreased body weight gain, leukopenia, liver toxicity, and induction of hepatic microsomal enzymes (Table 4-9). The data show that effects occurred primarily at concentrations ≥95 mg/kg-day for males and 126 mg/kg-day for females; however, liver lesions observed in one female mouse receiving 25 mg/kg-day aPCP are likely treatment related. Effects other than those listed in Table 4-9 are discussed below. Statistical analysis data were not reported for these effects. Rectal temperature was decreased by at least 1 degree in most groups of mice receiving all grades of PCP at 593 or 5,367 mg/kg-day in males and 645 or 3,852 in females. Urine color ranged from yellow to dark brown in males and females fed the mid and high doses of all PCP grades. Total liver porphyrins were increased in males receiving all three grades and in females receiving tPCP and aPCP. Uncoupling of mitochondrial oxidative phosphorylation (decreased phosphate:oxygen ratio) was observed at the high dose of aPCP, at the low dose of tPCP, and at the lower doses of EC-7 (<593 mg/kg-day for males or 645 mg/kg-day for females). The phosphate:oxygen ratio was increased at 593 mg/kg-day for males and at 645 mg/kg-day for females. The study authors did not determine NOAELs/LOAELs for the 30-day study. The EPA determined that the LOAELs were 95 mg/kg-day for males with all three grades of PCP, based on dose-related increases in liver lesions including hepatocyte degeneration and necrosis, centrilobular cytomegaly, karyomegaly, and nuclear atypia. For females, the LOAELs were 126 mg/kg-day for tPCP based on dose-related increases in liver lesions, 645 mg/kg-day for EC-7 based on liver lesions and decreased body weight gain, and 25 mg/kg-day for aPCP based on liver lesions. The NOAELs were 19 mg/kg-day in males for all grades and 25, 126, and 5 mg/kg-day in females for tPCP, EC-7, and aPCP, respectively.

Table 4-9. Comparison of the effects of three grades of PCP administered continuously in feed to male (M) and female (F) B6C3F$_1$ mice for 30 days

Effect[a]	tPCP (90.4% purity)	EC-7 (91.0% purity)	aPCP (98.6% purity)
Concentrations: 20, 100, 500, 2,500, 12,500 ppm average doses for males: 4, 19, 95, 593, 5,367 mg/kg-day; for females: 5, 25, 126, 645, 3,852 mg/kg-day			
Mortality	14/19 (M), 7/15 (F) at 12,500 ppm	19/19 (M), 5/5 (F) at 12,500 ppm 9/19 (M), 1/5 (F) at 2,500 ppm	19/19 (M), 5/5 (F) at 12,500 ppm 2/19 (M) at 2,500 ppm
Clinical signs	Weakness, lethargy, shallow breathing, severe weight loss, convulsions, and death at 12,500 ppm		
Body weight	Weight loss in both sexes, 12,500 ppm Decreased weight gain (M), 2,500 ppm	Decreased weight gain (M) at 2,500 ppm	Decreased weight gain in both sexes at 2,500 ppm
Liver weights	Absolute and relative weights statistically significantly increased at higher concentrations, both sexes		
Serum enzymes	ALP, cholesterol, ALT[b] increased in all animals, both sexes		
Serum γ-glutamyl transpeptidase (γ-GTP)	Greatly increased in both sexes at 2,500 and 12,500 ppm	No treatment-related increase	
Hematology	Clinically significantly marked reduction in leukocyte count, primarily affecting lymphocytes (M) and monocytosis (statistically significant in EC-7 females) in both sexes		
	Platelet count increased, both sexes	No increase in platelet count	
Hepatic microsomal enzymes	AHH[c] activity increased for both sexes, dose-related for tPCP; P450 levels increased in both sexes, dose-related for tPCP and aPCP		
Liver lesions[d]	≥500 ppm, 100% of animals of both sexes, more diffuse and severe than with other grades	≥500 ppm (M, 40%) ≥2,500 ppm (F, 100%)	≥500 ppm (M, 100%) ≥100 ppm (F, 100%)
LOAEL	500 ppm for both sexes 95 mg/kg-day (M). 126 mg/kg-day (F)	500 ppm, 95 mg/kg-day (M), 2,500 ppm, 645 mg/kg-day (F)	500 ppm, 95 mg/kg-day (M), 100 ppm, 25 mg/kg-day (F)
NOAEL	100 ppm for both sexes 19 mg/kg-day (M), 25 mg/kg-day (F)	100 ppm, 19 mg/kg-day (M), 500 ppm, 126 mg/kg-day (F)	100 ppm, 95 mg/kg-day (M), 20 ppm, 5 mg/kg-day (F)

[a]Statistical analyses were not reported for all effects.
[b]ALT = alanine aminotransferase.
[c]AHH = Aryl hydrocarbon hydroxylase.
[d]Centrilobular cytomegaly, karyomegaly, nuclear atypia, degeneration, or necrosis.

Source: NTP (1989).

Renner et al. (1987) reported on the toxicity of aPCP (99% purity) administered by gavage to rats for 4 weeks followed by 2 weeks of recovery. Groups of 24 female Sprague-Dawley rats (3 months old) were given 0.2 mmol/kg/day (53 mg/kg-day), 1 mL/day corn oil

(vehicle), or no treatment for the entire study duration. The results showed that body weights were not significantly affected by treatment with aPCP. No clinical signs were observed, but three aPCP-treated animals died on day 28 or 32 of the study. Relative liver weight was elevated during treatment, but returned to normal after treatment. Red blood cell (RBC), hematocrit, and hemoglobin were decreased throughout treatment and showed no evidence of reversal during recovery. The erythrocytes were polychromatic and anisocytotic in appearance. Microscopic effects in the liver consisted of enlarged pleomorphic hepatocytes with degeneration of liver cells and acidophilic bodies in the sinusoids. Statistical analysis was not reported. EPA determined the LOAEL was 53 mg/kg-day (the only dose used), based on decreased RBCs, hematocrit, and hemoglobin, and increased liver effects. The NOAEL could not be established as effects were noted at the only dose administered.

In a study on young, 6-week-old pigs, tPCP (purity not reported; contained 4.7% TCP and 3.2 ppm total OCDDs and -furans) was administered, in capsules at doses of 5, 10, or 15 mg/kg-day, to groups of six pigs (sex not reported) for 30 days (Greichus et al., 1979). No overt clinical signs or weight changes were noted in the tPCP-treated pigs compared with the controls. RBC parameters evaluated at 15 and 30 days showed no significant changes from controls. The white blood cell (WBC) count was significantly lower than control values for the 10 mg/kg-day dose group at 30 days and for the 15 mg/kg-day dose group at 15 and 30 days; values were near the lower limits of the normal range. The only serum chemistry change observed was significantly elevated blood urea nitrogen (BUN) in the 10 and 15 mg/kg-day dose groups after 15 days of treatment. The elevated BUN value, measured at study termination, for the 15 mg/kg-day dose group did not achieve statistical significance. The relative liver weights were significantly increased by 18 and 17% at 10 and 15 mg/kg-day, respectively. Histopathological findings in the liver of tPCP-treated pigs consisted of nonspecific cloudy swelling of hepatocytes accompanied by cellular enlargement, finely vacuolated cytoplasm, and decreased sinusoids. The investigators did not include incidence or severity of liver lesions for individual dose groups. Blood tPCP levels for all doses ranged from 63 to 71.5 ppm and from 67.6 to 78.1 ppm at 15 and 30 days of treatment, respectively, and no clear dose effect was observed. The highest tissue levels were measured in the liver and kidney followed by the muscle. The study authors did not determine NOAEL/LOAELs. The EPA determined that the LOAEL for pigs treated with tPCP for 30 days was 10 mg/kg-day, based on significantly increased relative liver weight accompanied by histopathological effects, significantly decreased WBC, and significantly increased BUN. The NOAEL was 5 mg/kg-day. The short-term oral studies for PCP are summarized in Table 4-10.

Table 4-10. Summary of effects and NOAELs/LOAELs for short-term studies on PCP

Species, strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Effect	Reference
Rat, F344 (10/sex/dose)	20, 40, 75, 150, or 270 (feed) 28 days	aPCP	20 (M)	40 (M)	Hepatocellular degeneration.	NTP, 1999
			40 (F)	75 (F)		
Rat, Sprague-Dawley (24 females)	53 (feed) 28 days	aPCP	NA	53	Decreased RBC, hematocrit, and hemoglobin. Polychromatic, and anisocytotic erythrocytes. Hepatocellular degeneration, enlarged pleomorphic hepatocytes, and acidophilic bodies in the sinusoids.	Renner et al., 1987
Mouse, $B6C3F_1$ (15/sex/dose for tPCP; 5/sex/dose for EC-7 and aPCP)	4, 19, 95, 593, or 5,367 (M) (feed) 30 days	tPCP	19	95	Liver lesions including hepatocellular degeneration and necrosis, centrilobular cytomegaly and karyomegaly, and nuclear atypia.	NTP, 1989
		EC-7				
		aPCP				
	5, 25, 126, 645, or 3,852 (F) (feed) 30 days	tPCP	25	126		
		EC-7	126	645		
		aPCP	5	25		
Pig (6/dose; sex not reported)	5, 10, or 15 (capsule) 30 days	tPCP	5	10	Increased relative liver weight, cloudy swelling of hepatocytes, finely vacuolated cytoplasm, decreased sinusoids, significantly elevated BUN, and decreased WBCs.	Greichus et al., 1979

[a]NOAELs and LOAELs determined by EPA for these studies; values for both genders unless otherwise specified.

4.2.1.2. *Subchronic Studies*

In a 6-month study conducted by NTP (1989), groups of 25 male and 10 female B6C3F$_1$ mice received either tPCP (90.4% purity) at 200, 600, or 1,800 ppm; EC-7 (91% purity) at 200, 600, or 1,200 ppm; DP-2 (91.6% purity) at 200, 600, or 1,200 ppm; or aPCP (98.6% purity) at 200, 500, or 1,500 ppm for 26–27 weeks. The average administered doses are estimated to be 38 and 301 mg/kg-day for males and 52 and 163 mg/kg-day for females fed 200 and 600 ppm tPCP, respectively. There was 100% mortality in the 1,800 ppm dose group and average doses could not be estimated. In animals fed 200, 600, or 1,200 ppm EC-7, the average doses are estimated for males as 36, 124, or 282 mg/kg-day and for females as 54, 165, or 374 mg/kg-day, respectively. The estimated average doses for 200, 600, or 1,200 ppm DP-2 are 40, 109, or 390 mg/kg-day for males and 49, 161, or 323 mg/kg-day for females, respectively. Males and females fed aPCP at dietary concentrations of 200, 500, or 1,500 ppm received estimated average doses of 102, 197, or 310 mg/kg-day for males and 51, 140, or 458 mg/kg-day for females, respectively. The estimated average dose administered to the low-dose group is much greater for those males fed aPCP than the other grades of PCP. The average doses were estimated by the EPA, using the feed intake values reported by NTP (1989). The intake for aPCP males in the low-dose group was much greater than the intake for the other dose groups, resulting in an estimated average dose that is approximately twofold greater than the other low-dose group animals. Statistical analyses were not reported for all effects.

Effects of administration of the four grades of PCP to mice for 6 months are summarized in Table 4-11. All groups of female mice receiving each grade of PCP had significantly increased absolute and relative liver weights. Groups of male mice receiving ≥38 mg/kg-day tPCP, ≥102 mg/kg-day aPCP, ≥109 mg/kg-day DP-2, and 282 mg/kg-day EC-7 also had significantly increased liver weights. Spleen weights were increased for all groups of male mice except the low dose of each grade, while spleen weights were significantly decreased in females at 163 mg/kg-day tPCP, 374 mg/kg-day EC-7, and 323 mg/kg-day DP-2. Thymus weights were not significantly affected. Liver lesions consisting of karyomegaly, cytomegaly, hepatocellular degeneration, and necrosis occurred in all males and females at all doses and grades of PCP. Liver pigmentation was observed in at least 6–10 males and females administered all doses of tPCP, the mid and high dose of DP-2 or EC-7, and the high dose of aPCP. Liver inflammation was observed in 8–10 high-dose male mice receiving tPCP, DP-2, and aPCP and in the females receiving tPCP. Bile duct hyperplasia occurred in all high-dose mice receiving tPCP. In addition, degenerative changes in the spleen, bone marrow, thymus, and testes occurred in animals that died before study termination. Effects observed with tPCP were generally more severe than those observed with other grades; however, nasal lesions were seen only with aPCP and EC-7. Other effects included dark urine color and elevated urine creatinine levels in high-dose males administered each grade and dark urine color in high-dose females administered

EC-7 and aPCP. In contrast to the 30-day study, rectal temperature was not elevated and leukocyte counts were not affected.

Table 4-11. Comparison of the effects of four grades of PCP administered continuously in feed to male (M) and female (F) B6C3F$_1$ mice for 6 months

Effect[a]	tPCP (90.4% purity) 200, 600, 1,800[b] ppm	EC-7 (91.0% purity) 200, 600, 1,200 ppm	DP-2 (91.6% purity) 200, 600, 1,200 ppm	aPCP (98.6% purity) 200, 500, 1,500 ppm
Estimated average dose	Males: 38 and 301 mg/kg-day Females: 52 and 163 mg/kg-day	Males: 36, 124, 282 mg/kg-day Females: 54, 165, 374 mg/kg-day	Males: 40, 109, 390 mg/kg-day Females: 49, 161, 323 mg/kg-day	Males: 102, 197, 310 mg/kg-day Females: 51, 140, 458 mg/kg-day
Mortality	100% (M, F) at 1,800 ppm; 0% at lower doses	1/10 (M) at 200 ppm; no other mortality observed	2/10 (M) at 1,200 ppm; no other mortality observed	2/20 (M) at 200 ppm; no other mortality observed
Clinical signs	Piloerection, hunched posture, enophthalmos, thinness, weakness, and inactivity at 1,800 ppm	None	Piloerection, hunched posture, enophthalmos, thinness, weakness, and inactivity at 1,200 ppm	None
Final body weights	No effect on survivors	11–13% decrease	No effect	No effect
Body weight gain	No effect on survivors	↓ at 1,200 ppm (M, F)	↓ at 1,200 ppm (M)	↓ at 1,500 ppm (M, F)
Serum enzymes				
ALT	Dose-related, statistically significant ↑ all animals, except EC-7 and DP-2 at 200 ppm			
AST[c]	Significant ↑ at 600 ppm (M, F)	No treatment-related ↑	Significant ↑ at 1,200 ppm (M)	Significant ↑ at 1,500 ppm (F)
γ-GTP	No effects (not reported for F)	No effects (not reported for F)	Significant ↑ at ≥600 ppm (M)	Significant ↑ at 1,500 ppm (M)
Liver weight	Significant ↑ at 200 and 600 ppm (M, F)	Significant ↑ at 1,200 ppm (M); ≥200 ppm (F)	Significant ↑ at 600 and 1,200 ppm (M); ≥200 ppm (F)	Significant ↑ all doses (M, F)
Hepatocellular lesions[d]	All doses, less severe in females than in males			
Liver pigment	All doses (M, F)	600 and 1,200 ppm (M, F)	600 and 1,200 ppm (M, F)	1,500 ppm (M, F)
Bile duct hyperplasia	All M and F at 1,800 ppm	No effect	No effect	No effect
Urinary bladder pigmentation	Minimal severity at all doses, less severe in females than in males receiving EC-7 or aPCP			
Nasal lesions[e]	No effect	≥600 ppm (M); all doses (F)	No effect	1,500 ppm (M); all doses (F)

Table 4-11. Comparison of the effects of four grades of PCP administered continuously in feed to male (M) and female (F) B6C3F$_1$ mice for 6 months

Effect[a]	tPCP (90.4% purity) 200, 600, 1,800[b] ppm	EC-7 (91.0% purity) 200, 600, 1,200 ppm	DP-2 (91.6% purity) 200, 600, 1,200 ppm	aPCP (98.6% purity) 200, 500, 1,500 ppm
Hepatic microsomal AHH induction	200 and 600 ppm (M)	1,200 ppm	All doses, maximum at 600 ppm	1,500 ppm
Hepatic P450 induction	200 and 600 ppm	1,200 ppm	All doses	1,500 ppm
LOAEL	200 ppm for all grades of PCP (approximately 38 mg/kg-day for tPCP, DP-2, and EC-7 and 102 mg/kg-day for aPCP males, respectively; approximately 52 mg/kg-day for all grades of PCP in females, based on liver lesions observed in all groups of mice tested			
NOAEL	None established; effects at all concentrations			

[a]Statistical analyses not reported for all effects.
[b]All animals in this group died and the estimated average doses could not be calculated.
[c]AST = aspartate aminotransferase.
[d]Cytomegaly, karyomegaly, degeneration, and necrosis.
[e]Nasal mucosal metaplasia and goblet cell hyperplasia.
↑ = increase; ↓ = decrease.

Source: NTP (1989).

The study authors did not determine the NOAELs/LOAELs for this subchronic study. The EPA determined that the LOAELs were 49-54 mg/kg-day for females for all four grades of PCP and at the low dose for males for all grades (36-40 mg/kg-day for tPCP, DP-2, and EC-7; 102 mg/kg-day for aPCP), based on dose-related increases in incidence and severity of liver lesions including hepatocellular degeneration and necrosis, karyomegaly, and cytomegaly. NOAELs were not established for males and females for any grade of PCP because liver toxicity was observed at all doses for all grades.

Kerkvliet et al. (1982a) administered 50, 250, or 500 ppm tPCP (average doses are estimated as 10, 51, or 102 mg/kg-day) to groups of six Swiss-Webster female mice in the diet for 8 weeks, followed by an 8-week recovery. Animals were sacrificed at 2-week intervals throughout treatment and recovery. Additionally, groups of 15–16 B6 female mice were administered 50, 100, or 250 ppm aPCP (average doses are estimated as 10, 20, or 49 mg/kg-day, respectively) for 8 weeks. No treatment-related effects were observed on body weights of either strain.

In the serial sacrifice study, relative liver weight, liver toxicity (hepatocyte swelling, nuclear swelling and vacuolization with eosinophilic inclusions in nuclear vacuoles, and mild to moderate multifocal necrosis), serum alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels in Swiss-Webster mice were elevated as early as 2 weeks after treatment with 51 mg/kg-day tPCP. Complete recovery occurred by 4–6 weeks after treatment was stopped. B6 mice exhibited significant increases in relative liver weight, liver toxicity, and decreases in thymus weight at doses of ≥20 mg/kg-day. Liver weights were significantly

increased at the mid (13–18%) and high (34–57%) doses for both strains. Thymus weights were reduced at the high dose for both strains, significantly for B6 mice at 49 mg/kg-day. The results of this aPCP study showed that effects on the liver can be caused by PCP alone in the absence of contaminants. The study authors did not determine the NOAELs/LOAELs. The EPA determined the LOAEL was 51 mg/kg-day for the tPCP-treated Swiss-Webster mice and 20 mg/kg-day for aPCP-treated B6 mice, based on dose-related increases in incidence and severity of multifocal necrosis, hepatocellular and nuclear swelling, hepatocellular vacuolization, and eosinophilic inclusion bodies in nuclear vacuoles. The NOAEL was 10 mg/kg-day for both tPCP- and aPCP-treated mice strains.

Kerkvliet et al. (1982b) reported that 20 male B6 mice/dose administered 50 or 500 ppm (average doses are estimated as 10 or 98 mg/kg-day) tPCP (86% purity) or aPCP (>99% purity) for 12 weeks showed no effects on growth rate, overt signs of toxicity, or microscopic changes in the kidney, spleen, or adrenal gland. However, dose-related mild to marked hepatocyte swelling was observed in the livers of animals exposed to both grades of PCP. Hepatocyte swelling, nuclear swelling, and vacuolization with eosinophilic inclusions in nuclear vacuoles were observed at 10 and 98 mg/kg-day. Mild to moderate multifocal necrosis was observed at 98 mg/kg-day. EPA determined that the LOAEL was 10 mg/kg-day, based on dose-related increases in hepatic effects. The NOAEL could not be determined as effects were noted at the lowest dose tested.

In a study conducted by Knudsen et al. (1974), 10 Wistar rat weanlings/dose/sex were fed diets containing 25, 50, or 200 ppm tPCP (average doses are estimated as 2, 5, or 18 mg/kg-day for males and 3, 5, or 21 mg/kg-day for females, respectively) for 12 weeks. The only biologically significant effects were a dose-related increase in aniline hydroxylase in liver microsomes and centrilobular vacuolation. Aniline hydroxylase activity was consistently increased at the low dose of males and females at 6 and 12 weeks, and significantly elevated in the 18 mg/kg-day male rats at 6 or 12 weeks and 21 mg/kg-day female rats at 6 weeks. The incidence of centrilobular vacuolation was increased in male rats at 5 (4/10) and 18 mg/kg-day (5/10) compared with 2/10 for the control and 0/10 for the 2 mg/kg-day group. The study authors determined that the LOAEL for this study was 5 mg/kg-day based on statistically significant increased incidence of liver effects; the NOAEL was 2 mg/kg-day for males and 3 mg/kg-day for females.

Johnson et al. (1973) described a study in which Sprague-Dawley rats (number of rats not reported) were fed diets containing three grades of PCP (described in general terms as commercial, improved, or chemically pure) for 90 days. None of these grades contained TCDD. The commercial PCP was 85–90% pure and contained 19 ppm hexachlorodibenzo-p-dioxin (HxCDD) and 1,980 ppm OCDD, the improved PCP was 88–93% pure and contained 1 ppm HxCDD and 26 ppm OCDD, and the chemically pure PCP (>99%) contained no detectable levels of chlorinated dioxins. The specific contaminant congeners were not identified. Treated

rats received PCP at doses of 3, 10, or 30 mg/kg-day. There were no effects on body weight with any of the three grades of PCP. Treatment with commercial PCP caused elevated serum ALP levels and liver and kidney weights at all concentrations. Serum albumin was increased at 10 and 30 mg/kg-day while erythrocyte count, hemoglobin concentration, and hematocrit were depressed at 30 mg/kg-day. Microscopic liver lesions (minimal focal hepatocellular degeneration and necrosis) were seen only at 30 mg/kg-day. The only effects observed after administering improved PCP and chemically pure PCP were elevated liver weight at 10 and 30 mg/kg-day and elevated kidney weight at 30 mg/kg-day. Quantitative changes and statistical analyses were not reported. The study authors did not determine NOAELs and LOAELs. The EPA determined that the LOAELs were 3 mg/kg-day (lowest dose tested) for commercial PCP based on dose-related elevated serum ALP and increased liver and kidney weight and 10 mg/kg-day for improved and pure PCP based on increased liver weight. The NOAEL was 3 mg/kg-day for improved and pure PCP, and could not be determined for commercial PCP.

Kimbrough and Linder (1975) reported light microscopic and ultrastructural effects in the liver of male rats (strain not specified) administered 1,000 ppm tPCP or aPCP (average dose estimated as 87 mg/kg-day) for 90 days. PCP treatment and control groups each consisted of 10 male rats. Statistical analysis was not reported. The liver was enlarged in all animals treated with PCP. Light microscopy revealed foamy cytoplasm or pronounced vacuolation of hepatocytes, single hepatocellular necrosis, cytoplasmic inclusions, slight interstitial fibrosis, prominent brown pigment in macrophages, and Kupffer cells in the livers of rats fed tPCP. Ultrastructurally, the smooth endoplasmic reticulum was increased, many lipid vacuoles were present, and the mitochondria had an atypical appearance. In rats fed aPCP, the hepatocytes were enlarged and many cells contained cytoplasmic inclusions; ultrastructurally, a slight increase in smooth endoplasmic reticulum, some lipid vacuoles, and atypical mitochondria were observed. This study showed that tPCP and aPCP cause similar ultrastructural effects in the liver. The study authors did not establish a LOAEL or NOAEL. The EPA determined that the LOAEL was 87 mg/kg-day for tPCP and aPCP, based on hepatocellular vacuolation, cytoplasmic inclusion, slight interstitial fibrosis, brown pigment in macrophages and Kupffer cells, and atypical mitochrondria. A NOAEL could not be determined.

Deichmann et al. (1942) administered tPCP in the diet to groups of 10 rats at a dose of 5 mg/day in 8.5 g of food for 26 weeks or 3.9 mg/day in 13 g of food for 28 weeks. The comparison group was not described. No growth occurred in rats administered 5 mg/day, and the rats receiving 3.9 mg/day had body weights below normal. No gross findings were noted for either group, and microscopic findings were considered insignificant.

Villena et al. (1992) examined the microscopic lesions in liver, kidney, and sciatic nerve of rats receiving PCP (grade not specified) for varied treatment times. Groups (number not reported) of male Wistar rats were given drinking water containing PCP at concentrations of 0.3 mM (80 mg/L) for 60 days, 1.0 mM (266 mg/L) for 60 or 90 days, 3.0 mM (800 mg/L) for

120 days, or drinking water without added PCP. The investigators did not describe effects in rats given 80 or 266 mg/L PCP for 60 days. Microscopic effects in the liver at 266 mg/L for 90 days or 800 mg/L for 120 days consisted of increased granular endoplasmic reticulum, hydropic vacuolar degeneration, and total cell degeneration (necrosis), congested portal veins, enlarged and congested sinusoids, and bile duct hyperplasia. The nephritis in the kidneys occurred primarily in the cortex and was characterized by glomerular congestion with thickening of the capillary wall, glomerular hyalinization, and hyaline casts in the lumen of the proximal convoluted tubules. The investigators noted that the kidney was more affected than the liver, and the effects imply that destruction could progress to loss of function in the kidney. The investigators did not state whether the animals were treated with free tPCP, aPCP, or sodium salts. This specific information is important considering that PCP has low solubility in water (80 mg/L) (Budavari et al., 1996), while the sodium salt is freely soluble in water. Additionally, effects on body weight, food, and water consumption, or clinical signs were not described. The authors did not establish a NOAEL or LOAEL. Based on the data presented in the report, the EPA determined the NOAEL was 80 mg/L and the LOAEL was 266 mg/L, based on dose-related increases in severity of liver and kidney toxicity.

Deichmann et al. (1942) reported no deaths or signs of toxicity in a group of 23 rabbits given 3 mg/kg of tPCP as a 1% aqueous solution (dosing method not reported) for 90 successive doses except on Sundays. In another study by Deichmann et al. (1942), five rabbits were administered tPCP orally at a dose of 35 mg/kg-day as a 0.5% solution for 15 days followed by a 5% solution to gradually increase the dose to 600 mg/kg-day (twice the lethal dose) during the next 19 days. All animals died, one after ingesting a total dose of 1.9 g, two after ingesting 2.9 g, and two after ingesting 3.9 g. Effects attributed to tPCP administration included weight loss and anemia.

McConnell et al. (1980) administered either 100% aPCP, 10% tPCP/aPCP mix, 35% tPCP/aPCP mix, or 100% tPCP to groups of three yearling (10–14 months) Holstein cattle to determine the effect of contaminants on PCP toxicity. The purity of PCP was not reported. Each treatment group was given 647 ppm PCP in feed (20 mg/kg) for 42 days, which was then decreased to 491 ppm (15 mg/kg) for the remaining 118 days of the study (total treatment time = 160 days). A group of three yearlings served as controls. The diet containing 100% tPCP produced more untoward effects than that of the 100% aPCP diet. Growth and feed efficiency were depressed by all PCP treatments but more severely by tPCP. The general appearance of tPCP-treated yearlings was unthrifty toward the end of the study. Yearlings receiving tPCP had a number of clinical and pathological abnormalities including anemia, increased hepatic mixed function oxidase and γ-glutamyl transpeptidase (γ-GTP) activities, increased relative liver and lung weights, thymus atrophy, and marked villous hyperplasia of the urinary bladder mucosa, which extended into the renal pelvis, renal papillae, and terminal portions of the collecting ducts (most striking lesion). Additionally, the yearlings exhibited signs of hyperplasia of the gall

bladder and bile duct mucosa, hyperkeratosis of ductal lining and dilated ducts containing keratinaceous material in the Meibomian glands in the eyelid, and hyperkeratosis of the skin. Many of these effects can be associated with exposure to dioxin and/or furan contaminants in PCP and were dose-related with respect to tPCP (i.e., the effects were more severe in cattle given 100% tPCP). In the 100% aPCP group, effects were limited to decreased concentrations of serum T_3 and thyroxine (T_4) and increased arylhydrocarbon hydroxylase (AHH) activity.

Kinzell et al. (1981) reported on the treatment of four lactating Holstein dairy cattle (6 weeks post partum) with dietary tPCP (85–90% purity). Cattle were given a dose of 0.2 mg/kg-day for 75–84 days followed by 2 mg/kg-day for an additional 56–60 days (total treatment time, 131–144 days). tPCP administration had no effect on body weight, food consumption, hematology, clinical chemistry, or urinalysis tests. Relative organ weights for liver, lung, kidney, and adrenals were increased by 23–27% compared with control (n = 4) weights; gross and microscopic lesions were observed in the kidney (chronic diffuse interstitial nephritis), and urinary bladder (thickening of bladder wall). In vitro tests revealed impairment of kidney function (decreased PAH, tetraethyl ammonium, and α-aminoisobutyrate uptake). These kidney effects were also observed in younger Holstein calves and attributed to PCP and not the contaminants (Hughes et al., 1985). No histopathologic effects attributable to tPCP were observed in the liver.

Hughes et al. (1985) fed tPCP (85–90% purity) or aPCP (99.02% purity) to 15 Holstein bull calves (7 days old) twice daily at doses of 0, 2, or 20 mg/kg-day. One calf in each of the high-dose groups fed aPCP or tPCP died after acute toxicity (elevated temperature, rapid respiration, severe diarrhea, acute purulent pneumonia). After 5 days, the doses of 2 and 20 mg/kg-day were lowered to 1 and 10 mg/kg-day, respectively, and treatment was continued for total treatment duration of 42 or 43 days. Severe toxic effects occurred following PCP administration, primarily in calves receiving tPCP. One calf treated with 10 mg/kg-day was moribund at the time of necropsy. Body weight gain, measured up to day 35 of treatment, was decreased in the 10 mg/kg-day dose groups when compared to that of controls. Body weight gain was decreased by 80 and 41% in calves receiving 10 mg/kg-day tPCP and aPCP, respectively. The overall marked decrease in weight was due primarily to a 93% decrease in weight gain for tPCP-treated calves relative to controls between days 20 and 35; the decrease for aPCP-treated calves was only 17%. Calves receiving 1 mg/kg-day of tPCP or aPCP gained slightly less weight than controls. During the last 3 weeks of treatment, tPCP-treated calves consumed only 15% as much grain as controls.

Thyroid hormone levels in serum were measured during the first 35 days of treatment. Serum T_3 levels were statistically significantly reduced by 58–69% after treatment with 10 mg/kg-day tPCP and 49–55% with 10 mg/kg-day aPCP. Treatment with 1 mg/kg-day reduced serum T_3 levels 44–56% with tPCP and 22–27% with aPCP. Reductions of 37–58 and 25% were observed in the calves' serum T_4 levels following treatment with 1 mg/kg-day tPCP

and aPCP, respectively. T_3 and T_4 responsiveness to the thyrotropin-releasing hormone (TRH) challenge were not affected by treatment with either grade. Organ weights most notably affected by PCP treatment were thymus and spleen in calves treated with 10 mg/kg-day tPCP or aPCP. The thymus weight was reduced by 83% with tPCP and 54% with aPCP. Microscopic lesions consistent with thymus atrophy (cortical atrophy) were observed in tPCP-treated calves. Spleen weights were reduced by 52% with 10 mg/kg-day tPCP and by 32% with 10 mg/kg-day aPCP. Squamous metaplasia was observed in the Meibomian gland of the eyelid of the three calves treated with 10 mg/kg-day tPCP, but in none of the calves treated with aPCP. The investigators attributed the eye effects to contaminants in PCP and not PCP itself. Statistically significantly elevated serum gamma-glutamyl transferase was observed with tPCP at 10 mg/kg-day. A decrease in serum protein concentration was noted at 10 mg/kg-day for both tPCP and aPCP.

In vitro tests to examine kidney function by observing p-aminohippurate and tetraethyl ammonium uptake indicated that 10 mg/kg-day PCP and not the contaminants impaired these energy-dependent functions. During treatment, Hughes et al. (1985) measured plasma PCP levels in calves. PCP levels rapidly increased then plateaued between 5 and 10 days. No difference was observed between the maximum plasma levels attained with tPCP and aPCP, although there were dose-related differences. The plasma PCP concentrations leveled off at approximately 100 ppm in calves given 10 mg/kg-day and at approximately 13–14 ppm in calves given 1 mg/kg-day. The PCP level in the plasma of control calves did not exceed 1 ppm. The authors did not establish NOAEL/LOAEL values. The EPA determined a NOAEL of 1 mg/kg-day and a LOAEL of 10 mg/kg-day, based on decreased body weight gain, significantly elevated serum gamma glutamyl transferase, decreased serum protein concentration, significantly decreased T_3 and T_4 levels, and decreased kidney function. The subchronic studies for PCP are summarized in Table 4-12.

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Table 4-12. Summary of NOAELs/LOAELs for oral subchronic studies for PCP

Species, strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Mice, Swiss-Webster (6 females/dose)	10, 51, or 102 (feed) 8 weeks	tPCP	10	51	Kerkvliet et al., 1982a[a]
Mice, B6 (15–16 female mice/dose)	10, 20, or 49 (feed) 8 weeks	aPCP	10	20	
Mice, B6 (20 males/dose)	10 or 98 (feed) 12 weeks	tPCP	NA	10	Kerkvliet et al., 1982b[a]
		aPCP			
Rat, Wistar weanlings (10/sex/dose)	2, 5, or 18 (M) 3, 5, or 21 (F) (feed) 12 weeks	tPCP	2 3	5 5	Knudsen et al., 1974
Rat, Sprague-Dawley (number not reported)	3, 10, or 30 (feed) 90 days	Commercial	NA	3	Johnson et al., 1973[a]
		Improved	3	10	
		Pure	3	10	
Rat (10 males/dose)	87 (feed) 90 days	tPCP	NA	87	Kimbrough and Linder, 1975[a]
		aPCP			
Rat, Male Wistar (number not reported)	80, 266, or 800 mg/L (drinking water) 60–120 days	Not reported	80	266	Villena et al., 1992[a]
Mice, B6C3F$_1$ (25 males/dose; 10 females/dose)	38 or 301 (M) (feed) 26–27 weeks	tPCP	NA (M)	38 (M)	NTP, 1989[a]
	52 or 163 (F) (feed) 26–27 weeks		NA (F)	52 (F)	
	36, 124, or 282 (M) (feed) 26–27 weeks	EC-7	NA (M)	38 (M)	
	54, 165, or 374 (F) (feed) 26–27 weeks		NA (F)	52 (F)	
	40, 109, or 390 (M) (feed) 26–27 weeks	DP-2	NA (M)	38 (M)	
	49, 161, or 323 (F) (feed) 26–27 weeks		NA (F)	52 (F)	
	102, 197, or 310 (M) (feed) 26–27 weeks	aPCP	NA (M)	102 (M)	
	51, 140, or 458 (F) (feed) 26–27 weeks		NA (F)	52 (F)	

[a]NOAELs and LOAELs determined by EPA for these studies; values for both genders unless otherwise specified.

4.2.1.3. *Chronic Studies—Noncancer*

In a chronic toxicity study in dogs (Mecler, 1996[1]), tPCP (90.9% purity) was fed by gelatin capsules to four beagle dogs/sex/dose at 0, 1.5, 3.5, or 6.5 mg/kg-day for 52 weeks. At 6.5 mg/kg-day, one male and one female dog were sacrificed in extremis on days 247 and 305, respectively, due to significant clinical toxicity (significant weight loss, lethargy, marked dehydration, vomiting, icterus). The morbidity was presumed due to hepatic insufficiency based on profuse toxicity in the liver that consisted of histologic lesions; multifocal, moderate hepatocellular swelling and degeneration of hepatocytes; fibrosis; bile duct hyperplasia; foci of hepatocellular hypertrophy; and hyperplasia consistent with cirrhosis. The mean body weight in surviving males in the 6.5 mg/kg-day dose group was decreased 18% when compared with controls. The decrease in body weight was not considered statistically significant as calculated by the study authors. Absolute body weight was only slightly decreased at the lower doses (4 and 6% at 1.5 and 3.5 mg/kg-day, respectively). Female dogs in the 6.5 mg/kg-day dose group exhibited a 20% decrease in absolute body weight that was statistically significantly less than controls at week 13 and for the remainder of the study. At the lower doses of 1.5 and 3.5 mg/kg-day, the absolute body weights of females were decreased 9 and 13%, respectively. In contrast to males, the decrease in absolute body weight in treated females was dose-related. Only group means were reported and individual animal data and standard deviations were not included.

There were dose-related mild to moderate decreases in three hematological parameters measured in male dogs for all dose groups, although not all changes were considered statistically significant (in calculations performed by study authors). Statistically significant decreases (15%) in red cell counts were observed in males at the 3.5 mg/kg-day dose, while the 1.5 mg/kg-day group showed only a 3% decrease. In males at the 6.5 mg/kg-day dose, RBC counts and hemoglobin levels were statistically significantly reduced by 21 and 16%, respectively, compared with controls. In females, statistically significant decreases of 10–17% in these hematological parameters were observed at 6.5 mg/kg-day from week 26 until study termination. In contrast to males, the hematological effects in females were not dose-related.

Activities of ALP, aspartate aminotransferase (AST), and ALT were elevated for both sexes throughout the study. At study termination, ALP activity was increased, compared with controls, in the serum of males (1.9-, 2.3-, and 4.9-fold) and females (1.9-, 2.6-, and 6.8-fold) at all three doses (1.5, 3.5, and 6.5 mg/kg-day, respectively). AST activity increased slightly at doses ≥3.5 mg/kg-day, although never more than 1.7-fold greater than in controls. The serum activity of ALT was similar to the control at 1.5 mg/kg-day, although ALT activity was observed

[1]This study was submitted to the Agency as part of the process for the development of the reregistration eligibility decision (RED) document by the U.S. EPA's Office of Pesticide Programs (OPP). Mecler (1996) satisfied the guideline requirements (OPPTS 870.4100) for a chronic toxicity study in non-rodents and is classified as an "acceptable" Good Laboratory Practice (GLP) study.

at levels 2.8- and 3.1-fold greater than the controls for males and females, respectively, in the 3.5 mg/kg-day dose group. Exposure to 6.5 mg/kg-day of PCP resulted in ALT levels 3.9- and 8.8-fold greater than in controls for males and females, respectively.

Male dogs exhibited increases of 10, 31, and 32% over controls in measurements of absolute liver weight at the 1.5, 3.5, and 6.5 mg/kg-day dose levels, respectively; these were not considered statistically significant by the study authors. However, increases of 14, 39, and 66% in relative liver weights of males were significantly greater than in controls in the 1.5, 3.5, and 6.5 mg/kg-day dose groups, respectively. Absolute and relative liver weights were significantly elevated at 1.5, 3.5, and 6.5 mg/kg-day doses in females by 24, 22, and 49% (absolute liver weights) and 37, 40, and 94% (relative liver weights), respectively. Thyroid weight measurements in males were increased when compared with controls, but did not show a linear dose-response relationship. Absolute and relative thyroid weights were statistically significantly increased in females at the 6.5 mg/kg-day dose by 78 and 138%, respectively. Relative thyroid weight was also increased at the 1.5 (72%) and 3.5 mg/kg-day (64%) doses.

An increased incidence of gross stomach lesions consisting of multiple, raised mucosal foci were observed in all treated groups (1.5, 3.5, and 6.5 mg/kg-day) of male (2/4, 3/4, and 2/3, respectively, versus 0/4 in controls) and female (2/4, 4/4, and 2/3, respectively, versus 1/4 in controls) dogs. Male dogs exhibited dark, discolored livers in 1/4, 1/4, and 3/3 dogs, while 3/4, 3/4, and 2/3 females exhibited the discolored livers in the 1.5, 3.5, and 6.5 mg/kg-day treatment groups, respectively. Microscopically, liver lesions associated with tPCP treatment consisted of pigmentation, cytoplasmic vacuolization, minimal necrosis, and chronic inflammation; incidence and severity generally increased with dose. The incidence and severity of the liver lesions in male and female dogs are shown in Table 4-13. The authors noted that the pigmentation was approximately 2–4 microns in diameter and segregated near the cytoplasmic membrane. The pigment was sometimes observed in the regions of canaliculi of adjacent hepatocytes, and less frequently in the cytoplasm of Kupffer cells and histiocytes within periportal regions. The authors considered the pigment consistent with lipofuscin, noting that biotransformation of chlorinated phenolic compounds occurs via CYP450 enzymes, during which time lysosome-related peroxidation of intracellular lipids produces lipofuscin pigment. The study authors determined that the LOAEL was 6.5 mg/kg-day tPCP, based on morphologic effects in the liver. The NOAEL was 3.5 mg/kg-day. However, considering the progression of lesions observed with increasing dose and the morbidity observed in both sexes at the 6.5 mg/kg-day dose, the EPA determined that the LOAEL was 1.5 mg/kg-day (lowest dose tested), based on liver pathology consisting of dose-related increases in incidence and severity of hepatocellular pigmentation, cytoplasmic vacuolation, and chronic inflammation, and significant increases in relative liver weight and increases in absolute liver weight (significant in females), and increased serum enzyme activity. The NOAEL could not be established.

Table 4-13. Liver histopathology, incidence, and severity in dogs exposed to tPCP

Dose (mg/kg-day)	Females				Males			
	0	1.5	3.5	6.5	0	1.5	3.5	6.5
Number examined	4	4	4	3	4	4	4	3
Lesion[a]								
Pigment	0	4 (2.3)	4 (2.8)	3 (3.3)	0	4 (3)	4 (3)	3 (3.3)
Cytoplasmic vacuolization	3 (1)	3 (2)	4 (2.3)	3 (3.3)	1 (3)	1 (2)	4 (2.8)	3 (3.3)
Minimum necrosis	0	0	0	2 (1)	0	0	0	1 (1)
Chronic inflammation	2 (1)	2 (1.5)	4 (1.8)	3 (1.7)	0	4 (1)	4 (1.3)	3 (1.3)

[a]The values in parentheses are grades of severity for the lesion: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Source: Mecler (1996).

In a study conducted by NTP (1989), groups of 50 $B6C3F_1$ mice/sex/dose were administered feed containing 100 or 200 ppm tPCP (90.4% purity) or 100, 200, or 600 ppm EC-7 (91% purity) continuously for 2 years. Two groups of mice (35 animals/sex) were maintained on untreated feed to serve as controls. The average administered dose in the treated feed was calculated as 18 or 35 mg/kg-day for males and 17 or 35 mg/kg-day for females for the 100 or 200 ppm dose groups, respectively, for tPCP or 18, 37, or 118 mg/kg-day for males, and 17, 34, or 114 for females for the 100, 200, or 600 ppm dose groups, respectively, for EC-7. Both tPCP and EC-7 contain approximately 90% PCP, but different levels of contaminants. The average daily PCP and contaminant doses associated with each dietary concentration are summarized in Table B-3 in Appendix B. Mean body weights of male and female mice receiving either tPCP or EC-7 were similar to control weights throughout the study with one exception. Female mice receiving 114 mg/kg-day EC-7 weighed 78–91% of the control weights during the second year of the study. No statistically significant effects were observed on survival in either male or female mice receiving tPCP or EC-7, although the survival rate of tPCP male controls was abnormally low (34%) at the end of the study.

This study showed that the liver was the primary target for systemic toxicity for both grades of PCP and in both sexes. The following liver lesions occurred at statistically significant higher incidences in PCP-treated males at all doses of tPCP and EC-7 than in the control: clear cell focus, acute diffuse necrosis, diffuse cytomegaly, diffuse chronic active inflammation, multifocal accumulation of brown pigmentation (lipofuscin [LF] and cellular debris) in Kupffer cells, and proliferation of hematopoietic cells (extramedullary hematopoiesis). Males also had a significantly higher incidence of bile duct hyperplasia at both doses of tPCP, but only at the 114 mg/kg-day dose of EC-7. Females receiving all doses of tPCP and EC-7 exhibited incidences of the following liver lesions that were significantly higher than controls: cytomegaly, necrosis, inflammation, and pigment accumulation. In addition, the incidence of

clear cell focus was significantly increased compared with controls in females treated with 17 mg/kg-day tPCP and 34 and 114 mg/kg-day EC-7. The incidence of extramedullary hematopoiesis was higher in females exposed to 35 mg/kg-day tPCP and all doses of EC-7 when compared with that in controls. In contrast to males, the female mice did not exhibit a significant increase in bile duct hyperplasia with tPCP, although the hyperplasia was significantly higher in females treated with 114 mg/kg-day EC-7. This was the only lesion that the investigators related solely to the impurities within PCP.

Other treatment-related nonneoplastic findings were observed in the spleen and nose of male and female mice and in the mammary glands of females. The incidence of extramedullary hematopoiesis in the spleen was significantly higher in tPCP males at 18 and 35 mg/kg-day and in females at 35 mg/kg-day. Acute focal inflammation of the mucosal gland and focal metaplasia of the olfactory epithelium were increased in male (118 mg/kg-day) and female mice (114 mg/kg-day) receiving EC-7; these lesions did not occur in any mouse receiving tPCP. In tPCP females, the incidence of cystic hyperplasia of the mammary gland was significantly higher at 35 mg/kg-day (59%) than in tPCP controls (23%) but not when compared with the EC-7 control (58%). Therefore, this lesion was not considered related to treatment by investigators. Under the conditions of these studies, tPCP and EC-7 were equally effective in male mice except for induction of bile duct hyperplasia. In female mice, tPCP was generally more effective than EC-7 except for induction of bile duct hyperplasia and nasal lesions. The study authors did not determine LOAELs/NOAELs. The EPA determined that the LOAELs were 18 mg/kg-day for males and 17 mg/kg-day for females for both tPCP and EC-7, based on statistically significant increases in liver lesions. NOAELs could not be established for either tPCP or EC-7, because effects in the liver occurred at the lowest doses tested in male and female mice. Some findings occurred at incidences approaching 100% at 100 ppm (17–18 mg/kg-day), indicating that a lower dose could have been tested and the potential for low-dose toxicity exists.

In a chronic toxicity study, Schwetz et al. (1978) administered DOWICIDE EC-7, a commercial-grade PCP (91% purity) in the diet of male and female Sprague-Dawley rats at doses of 0, 1, 3, 10, or 30 mg/kg-day. Treated or control diets were fed to males for 22 months and females for 24 months. Each group consisted of 25 rats of each sex. Statistical analysis was not reported. No treatment-related effects were observed for clinical signs, food consumption, survival, hematological parameters, or organ weights. The investigators stated that mean body weights of high-dose females were significantly less than those of controls during most of the study. Serum ALT activity was slightly increased (<1.7-fold) in both sexes at the highest dose when measured at study termination. Histopathological examination showed pigment accumulation in the centrilobular hepatocytes of the liver in 30% of females given 10 mg/kg-day and in 59% of females given 30 mg/kg-day. Similarly, 26 and 70% of females receiving 10 and 30 mg/kg-day EC-7 exhibited pigment accumulation in the epithelial cells of the proximal convoluted tubules in the kidney. This effect was not detected in the females of the lower dose

or control groups. Only 1 of the 27 male rats given EC-7 (30 mg/kg-day) exhibited the brown pigment in hepatocytes. The study authors determined that the LOAEL was 30 mg/kg-day for males and 10 mg/kg-day for females, based on dose-related increased pigment accumulation in the liver and kidney. The NOAELs were 10 mg/kg-day for males and 3 mg/kg-day for females.

Kimbrough and Linder (1978) compared the effect of tPCP (84.6%) and aPCP (>99%) fed to male and female Sherman rats for 8 months, observing that effects following administration of tPCP were more severe than those of aPCP. PCP was administered at concentrations of 20, 100, or 500 ppm (average doses are estimated as 2, 9, or 44 mg/kg-day for males and 2, 10, or 48 mg/kg-day for females, respectively). No signs of mortality were observed with either tPCP or aPCP. Final body weights were significantly reduced 15–16% for both male and females fed the high dose of tPCP and 5 and 10% for females and males, respectively, fed the high dose of aPCP. Dose-related effects were observed in the liver, particularly in rats fed tPCP (effects were described qualitatively; the quantitative changes were not reported). Liver weights were elevated in both sexes (statistically significant in the males) at the high dose of tPCP. Animals treated with 44 (males) or 48 mg/kg-day (females) tPCP exhibited liver toxicity (statistical analyses not reported), manifested by periportal fibrosis, hepatocyte hypertrophy, vacuolation, pleomorphism, bile duct proliferation, adenofibrosis (cholangiofibrosis), cytoplasmic hyaline inclusions, and abundant brown pigment in macrophages and Kupffer cells (porphyria) in one or both sexes. At 9 (males) or 10 mg/kg-day (females) tPCP, similar but less severe effects than those observed at the high doses were observed, although adenofibrosis and bile duct proliferation did not occur at this dose. A small neoplastic nodule was observed in the liver of one mid-dose female rat. At the lowest dose of 2 mg/kg-day tPCP, slight hepatocyte hypertrophy and vacuolation were observed in all males and one female. In rats administered aPCP at doses of 44 (males) and 48 mg/kg-day (females), effects in the liver included slight hepatocyte hypertrophy, eosinophilic cytoplasmic inclusions, and brown pigment in macrophages in animals of one or both sexes. There were no effects observed in rats treated with the two lower doses of aPCP. The EPA determined that the LOAELs were 2 mg/kg-day (lowest dose tested) for tPCP and 44 mg/kg-day in males and 48 mg/kg-day in females for aPCP, based on dose-related increases in incidence and severity of liver effects and statistically significant decreases in body weight. The NOAEL could not be determined for tPCP. The NOAELs were 9 and 10 mg/kg-day for males and females, respectively, for aPCP.

NTP (1999) examined groups of 50 F344 rats/sex/dose administered aPCP (99% purity, with no detectable levels of chlorinated dibenzo-p-dioxin, dibenzofuran, diphenyl ether, or hydroxydiphenylether) in feed at concentrations of 0, 200, 400, or 600 ppm (average doses of 0, 10, 20, or 30 mg/kg-day, respectively) for 105 weeks. In an additional stop-exposure study, groups of 60 rats/sex were maintained on feed containing 1,000 ppm aPCP (average dose of 60 mg/kg-day) for 52 weeks followed by untreated feed until study termination at 2 years. This

study was also reported by Chhabra et al. (1999). Survival rates of male rats receiving 30 mg/kg-day for 2 years or 60 mg/kg-day for 52 weeks significantly exceeded those of controls (62 or 64%, respectively, versus 24% for controls), while survival of the other groups was similar to that of controls. Mean body weights were decreased in both male and female rats at various times during the study. Mean body weights were 94, 91, 89, and 82% of the control weights in males and 94, 91, 84, and 78% of the control weights in females receiving 10, 20, 30, and 60 mg/kg-day aPCP, respectively. In the stop-exposure study, body weights recovered to within 4% of the control weight after treatment stopped at 52 weeks.

The liver was the primary target for nonneoplastic toxicity, particularly in male rats. The incidence of cystic degeneration was significantly increased at 20 (56%) and 30 (78%) mg/kg-day. In addition, the incidence of hepatodiaphragmatic nodules was significantly increased in all groups of males receiving aPCP (10–16 versus 0% for controls), although no clear dose-response was observed. Hepatodiaphragmatic nodules were described as developmental anomalies commonly observed in F344 rats; therefore, the increased incidence observed in this study was not considered related to exposure to aPCP. The incidences of liver lesions in female rats in the 2-year study were similar to or significantly lower than those of controls (cytoplasmic hepatocyte vacuolation in 2 versus 14% for controls).

Interim evaluation (7 months) of the stop-exposure group exhibited significantly elevated (20–90%) serum ALP levels in males and sorbitol dehydrogenase levels in males and females compared with control levels. The ALT level in males was elevated by 46%, but this was not statistically significant as calculated by the investigators. Microscopic examination of 60 mg/kg-day rats, sacrificed at 7 months, showed significantly higher incidences of centrilobular hepatocyte hypertrophy in both male and female rats (60%) and cytoplasmic hepatocyte vacuolization in male rats (80%) compared with the controls (0%). These microscopic lesions were also observed in male and female rats of the 2-year study; however, incidences were not significantly increased. The 60 mg/kg-day males exhibited a significantly greater incidence, compared with controls, of liver lesions consisting of chronic inflammation (64 versus 44% for controls), basophilic focus (62 versus 34% for controls), and cystic degeneration of hepatocytes (56 versus 32% for controls). The study authors did not determine LOAELs and NOAELs. This study showed that male rats were more susceptible to aPCP exposure than female rats with one exception; males and females were equally responsive to aPCP in the stop-exposure study. The EPA determined that the LOAEL was 20 mg/kg-day for male rats based on statistically significant increases in cystic degeneration; the NOAEL was 10 mg/kg-day. The LOAEL was 30 mg/kg-day for female rats based on a biologically significant decrease in body weight; the NOAEL was 20 mg/kg-day. The chronic studies for PCP are summarized in Table 4-14.

Table 4-14. Summary of NOAELs/LOAELs for oral chronic studies for PCP

Species	Dose (mg/kg-day)/ duration	Grade/Type of PCP	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Rat, Sherman (10/sex/dose)	2, 9, or 44 (M) 2, 10, or 48 (F) 8 months (Feed)	aPCP	9 (M) 10 (F)	44 (M) 48 (F)	Kimbrough and Linder, 1978[a]
	2, 9, or 44 (M) 2, 10, or 48 (F) 8 months (Feed)	tPCP	NA	2	
Dog, beagle (4/sex/dose)	1.5, 3.5, or 6.5 1 year (Gelatin capsule)	tPCP	NA	1.5	Mecler, 1996[a]
Rat, F344 (50/sex/dose)	10, 20, or 30 2 years (Feed)	aPCP	10 (M) 20 (F)	20 (M) 30 (F)	NTP, 1999 [a]
Rat, Sprague-Dawley (25/sex/dose)	1, 3, 10, or 30 2 years (Feed)	EC-7	10 (M) 3 (F)	30 (M) 10 (F)	Schwetz et al., 1978
Mouse, $B6C3F_1$ (50/sex/dose)	18 or 35 (M) 17 or 35 (F) 2 years (Feed)	tPCP	NA	18 (M) 17 (F)	NTP, 1989[a]
	18, 37, or 118 (M) 17, 34, or 114 (F) 2 years (Feed)	EC-7	NA	18 (M) 17 (F)	

[a]NOAELs and LOAELs determined by EPA for these studies; values for both genders unless otherwise specified.

4.2.2. Inhalation Studies

4.2.2.1. *Subchronic Studies*

No subchronic inhalation studies that examined the effects of PCP in humans are available. A Chinese study (Ning et al., 1984; translation) exposed weanling male rats to 3.1 or 21.4 mg/m^3 PCP (reagent grade, Na-PCP) 4 hours/day, 6 days/week, for 4 months. Rats in the 21.4 mg/m^3 group exhibited significant increases, compared with control, in lung, kidney, liver, and adrenal gland weight. Additionally, the levels of blood-glucose were elevated in rats exposed to the high concentration of PCP. Ning et al. (1984) also observed statistically significantly increased serum γ-globulin (although not α-globulin, β-globulin, or serum albumin) and lung and liver weights in six rabbits (pooled males and females) exposed, in a similar manner, to 21.4 mg/m^3. Demidenko (1969) reported results in which anemia, leukocytosis, eosinophilia, hyperglycemia, and dystrophic processes in the liver were observed in rats and rabbits exposed to 28.9 mg/m^3 PCP (high concentration; purity not reported) for 4 hours/day for 4 months. Animals exposed to the low concentration (2.97 mg/m^3) exhibited effects on liver function, cholinesterase activity, and blood sugar that were considered minor and were not observed 1 month following exposure completion. Kunde and Böhme (1978), calculated an estimated dose of 0.3 mg/kg-day PCP based on the 2.97 mg/m^3 concentration reported by Demidenko (1969). This calculation assumed 100% pulmonary uptake and absorption.

4.2.2.2. *Chronic Studies*

No chronic inhalation studies that examined the effects of PCP in humans or animals are available.

4.2.3. Other Routes of Exposure

A 13-week dermal toxicity study was conducted in groups of 10 male and 10 female Sprague-Dawley rats/dose receiving 0, 100, 500, or 1,000 mg/kg-day doses of tPCP (88.9% purity) applied to clipped dorsal skin for 6 hours/day for 91 days (Osheroff et al., 1994). tPCP, applied without a vehicle, was held in place by a gauze patch. Some degree of skin irritation (acanthosis and chronic inflammation) was observed in both sexes at all doses of tPCP. Chronic inflammation was observed in 10, 80, and 100% of males and 0, 100, and 100% of females treated with 100, 500, and 1,000 mg/kg-day tPCP, respectively. Hepatocellular degeneration was observed in 90 and 100% of males at the mid and high doses, respectively, and in 20, 100, and 100% of females in the low, mid and high doses, respectively. ALT was statistically significantly increased 4.3- and 7.6-fold in males and 2.5- and 5.4-fold in females in the 500 and 1,000 mg/kg-day dose groups, respectively, and AST was statistically significantly increased 2.3- and 3.3-fold in males and 1.8- and 3.1-fold in females in the 500 and 1,000 mg/kg-day dose groups, respectively. Relative liver weights were statistically significantly increased over controls in the 100 (11%), 500 (18%), and 1,000 (30%) mg/kg-day dose groups for male rats. In females, the relative liver weights in animals of the 500 (18%) and 1,000 (36%) mg/kg-day dose groups were significantly greater than controls. Additionally, relative kidney weights were increased 20% in 1,000 mg/kg-day males and 56 and 16% in 500 and 1,000 mg/kg-day females, respectively. This study showed that PCP is absorbed from the skin at levels that caused liver toxicity. The study authors determined that the LOAEL for this study was 500 mg/kg-day based on dose-related increases in liver toxicity (hepatocellular degeneration, chronic inflammation, and statistically significant increases in hepatic enzyme induction). The NOAEL was 100 mg/kg-day.

4.2.4. Cancer Studies

4.2.4.1. *Oral Studies*

NTP (1989) administered feed containing 100 or 200 ppm tPCP (90.4% purity) or 100, 200, or 600 ppm EC-7 (91% purity) to B6C3F$_1$ mice (50/sex/group) continuously for 2 years (NTP, 1989). Two groups of 35 mice of each sex maintained on untreated feed served as controls for each grade of PCP. The average daily doses were estimated as 18 and 35 mg/kg-day for 100 and 200 ppm tPCP males, respectively, and 17 and 34 mg/kg-day for 100 and 200 ppm tPCP females, respectively. The doses of EC-7 administered to male and female mice were estimated as 18, 37, or 118 mg/kg-day for males, and 17, 34, or 114 for females, respectively. The average daily PCP and contaminant doses associated with each dietary concentration are summarized in Table B-3 of Appendix B. Statistical analyses included the Life Table Test that

considered tumors as fatal in animals dying before study termination, the Logistic Regression Test that regarded all lesions as nonfatal, and the Fisher Exact and Cochran-Armitage Trend Test that compared the overall incidence rates of treated groups with controls. Nonneoplastic findings are discussed in Section 4.2.1.

The incidences of treatment-related tumors and results of the statistical analyses are presented in Tables 4-15 (males) and 4-16 (females). In male mice, the incidence of hepatocellular adenoma and carcinoma were statistically significantly elevated by both grades of PCP compared with controls. The incidence of hepatocellular adenoma was statistically significantly elevated in males receiving 18 mg/kg-day tPCP diet (43 versus 16% for controls), but not in males receiving the 18 mg/kg-day EC-7 diet (27 versus 14% for controls). The incidence of hepatocellular carcinoma in males was only marginally statistically increased (p = 0.06 or 0.07) by both grades at 18 mg/kg-day (21% in tPCP and 15% in EC-7), although the incidence was statistically significantly increased at 35 mg/kg-day for tPCP (25%) and at 37 mg/kg-day for EC-7 (15%) when compared with individual control groups. However, the incidence of hepatocellular carcinoma in the 18 mg/kg-day dose groups was statistically significantly (p = 0.006) elevated when compared with the combined control groups. The incidence of hepatocellular adenoma/carcinoma was statistically significantly increased with all doses of tPCP and EC-7. The incidences were greater in male mice receiving tPCP (55 and 77% at 18 and 35 mg/kg-day, respectively) than in males receiving EC-7 (40, 44, and 69% at 18, 37, and 118 mg/kg-day, respectively). In female mice, the incidence of hepatocellular adenoma (63%) was statistically significantly elevated only at the 114 mg/kg-day dose of EC-7 when compared with the control group, and the incidence of hepatocellular carcinoma (range of 2–4%) was not significantly elevated in females treated with either grade of PCP. If incidence of hepatocellular adenoma in female groups treated with tPCP is compared with the combined control groups, then statistical significance is achieved at 17 mg/kg-day (p = 0.05; 16%) with marginal significance at 34 mg/kg-day (p = 0.06; 16%).

Table 4-15. Treatment-related tumors in male B6C3F$_1$ mice fed tPCP or Dowicide EC-7 for 2 years

	tPCP			Dowicide EC-7			
Organ/lesions[a]	**Control**	**18 mg/kg-day**	**35 mg/kg-day**	**Control**	**18 mg/kg-day**	**37 mg/kg-day**	**118 mg/kg-day**
Liver—hepatocellular							
Adenoma	5/32	20/47[c,d]	33/48[b,c,d]	5/35	13/48	17/48[b,c,d]	32/49[b,c,d]
Carcinoma	2/32	10/47	12/48[c,d]	1/35	7/48	7/48[b,c]	9/49[b,c,d]
Adenoma/carcinoma	7/32	26/47[c,d]	37/48[b,c,d]	6/35	19/48 [b,c,d]	21/48[b,c,d]	34/49[b,c,d]
Adrenal gland/medulla							
Pheochromocytoma				0/34	4/48	21/48[b,c,d]	44/49[b,c,d]
Malignant pheochromocytoma				1/34	0/48	0/48	3/49
Pheochromocytoma/ malignant	0/31	10/45[b,c,d]	23/45[b,c,d]	1/34	4/48	21/48[b,c,d]	45/49[b,c,d]

[a]Data reported as number of animals with tumors/number of animals examined at the site.
[b]Statistically significant as calculated by Life Table Analysis.
[c]Statistically significant as calculated by Logistic Regression Test.
[d]Statistically significant as calculated by the Cochran-Armitage Trend or Fisher Exact Test.
[e]No statistical analyses reported.

Source: NTP (1989).

Table 4-16. Treatment-related tumors in female B6C3F$_1$ mice fed tPCP or Dowicide EC-7 for 2 years

Organ/lesions[a]	tPCP			Dowicide EC-7			
	Control	17 mg/kg-day	35 mg/kg-day	Control	17 mg/kg-day	34 mg/kg-day	114 mg/kg-day
Liver—hepatocellular							
Adenoma	3/33	8/49	8/50	1/34	3/50	6/49	30/48[b,c,d]
Carcinoma	0/33	1/49	1/50	0/34	1/50	0/49	2/48
Adenoma/carcinoma	3/33	9/49	9/50	1/34	4/50	6/49	31/48[b,c,d]
Adrenal gland/medulla							
Pheochromocytoma				0/35	1/49	2/46	38/49[b,c,d]
Malignant pheochromocytoma[e]				0/35	1/49	0/46	1/49
Pheochromocytoma/malignant	2/33[e]	2/48[e]	1/49[e]	0/35	2/49	2/46	38/49[b,c,d]
Circulatory system							
Hemangioma[e]				0/35	0/50	0/50	1/49
Hemangiosarcoma	0/35	3/50	6/50[b,c,d]	0/35	1/50	3/50	9/49[b,c,d]
Hemangioma/hemangiosarcoma				0/35	1/50	3/50	9/49[b,c,d]

[a]Data reported as number of animals with tumors/number of animals examined at the site.
[b]Statistically significant as calculated by Life Table Analysis.
[c]Statistically significant as calculated by Logistic Regression Test.
[d]Statistically significant as calculated by the Cochran-Armitage Trend or Fisher Exact Test.
[e]No statistical analyses reported.

Source: NTP (1989)

Adrenal gland medullary pheochromocytomas occurred in 22 and 51% of male mice receiving 18 and 35 mg/kg-day tPCP, respectively, and in 44 and 90% of male mice receiving 37 and 118 mg/kg-day EC-7, respectively, but in none of the controls. Pheochromocytomas also developed in 78% of females receiving 114 mg/kg-day compared with only one or two female mice in the control groups or 17 and 34 mg/kg-day dose groups. Hemangiosarcomas, which developed primarily in the liver and spleen, were observed in 6 and 12% of females receiving 17 and 34 mg/kg-day tPCP, and 2, 6, and 18% receiving 17, 34, and 114 mg/kg-day EC-7, and none in the 70 controls examined. Hemangiosarcomas were also observed in male mice administered both grades of PCP, although the incidences were low (4-6% in tPCP-exposed mice and 6-10% in EC-7-exposed mice vs 3% in control) and were not statistically significantly different from the control.

The results of this study show that tumors were induced in mice exposed to tPCP and EC-7. The latter contains relatively low levels of dioxin and furan impurities compared to tPCP. Based on tumor response, tPCP was slightly more potent. NTP (1989) and McConnell et al. (1991) compared the concentrations of HxCDD, a known contaminant of PCP, in tPCP and EC-7 with that known to induce liver tumors in mice and concluded that the carcinogenic response in mice can be attributed primarily to PCP and that the impurities provided a minor contribution. NTP (1989) concluded that PCP is primarily responsible for the carcinogenicity observed in mice and that impurities played only a small part in the neoplastic process, at least in the liver of male mice. NTP further concluded that there was *clear evidence of carcinogenic activity* for male mice receiving tPCP and male and female mice receiving EC-7 and *some evidence of carcinogenic activity* for female mice receiving tPCP.

Bionetics Research Labs (BRL), Inc. (BRL, 1968) carried out two long-term (18-month) studies of EC-7 (90% purity) in $B6C3F_1$ and B6AKF1 mice, one using continuous oral administration and the other a single subcutaneous injection. In the first study, mice (18 mice/sex/strain) were exposed to EC-7 by gavage (in 0.5% gelatin) at a dose of 46.4 mg/kg-day starting on day 7 of age through weanling (day 28 of age). Thereafter, mice received EC-7 in the diet at a dose initially corresponding to 46.4 mg/kg-day; dosing continued for up to 18 months of total exposure. No adjustments to the dietary concentration were made for body weight gain during the study. In the second experiment, 28-day-old mice of the same strains (18 mice/sex/strain) received a single, subcutaneous injection of 46.4 mg/kg EC-7 in the neck and were examined at 18 months. Male and female mice exposed to EC-7 in this study did not develop tumors that were considered statistically significantly greater in incidence than tumors observed in control animals.

In the NTP (1999) study, groups of 50 male and 50 female F344 rats were administered aPCP (99% purity) in feed at concentrations of 0, 200, 400, or 600 ppm continuously for 105 weeks; additional groups of 60 male and 60 female rats were maintained on feed containing 1,000 ppm aPCP for 52 weeks followed by untreated feed until study termination at 2 years in a

stop-exposure study. The average doses of PCP were reported as 10, 20, 30, and 60 mg/kg-day for male and female rats fed the 200, 400, 600, and 1,000 ppm diets, respectively. Histopathologic examination showed a statistically significantly higher incidence (18%) of malignant mesothelioma in 60 mg/kg-day males compared with controls; the incidence exceeded the range of historical controls. The mesotheliomas originated from the tunica vaginalis. The incidence of nasal squamous cell carcinomas was also elevated (10%) in 60 mg/kg-day males. At study termination (2 years), the nasal tumors spread to the oral cavity in one of the male rats in this dose group. When compared with concurrent controls, the tumor incidence in male rats did not achieve statistical significance but did exceed the range of historical controls. Nasal squamous cell carcinoma at 10 mg/kg-day was the only neoplastic finding in male rats treated for the entire 2 years that occurred with a higher incidence (6%) than that of historical controls. However, NTP (1999) did not consider the finding at 10 mg/kg-day to be treatment related because the incidence at 20 (2%) and 30 mg/kg-day (0%) was less than or no greater than that of concurrent controls (2%). Therefore, the only treatment-related tumors that occurred in male rats were in those animals exposed to 60 mg/kg-day PCP in the stop-exposure study. The tumors observed in the stop-exposure study were observed earlier than tumors at other doses (45 days earlier for nasal tumors and 91 days earlier for mesotheliomas) and did not regress during the observation year in which animals were administered untreated feed. There were no treatment-related increases in tumor incidence at any site in females receiving aPCP. These data and results of the statistical analyses are presented in Table 4-17. NTP concluded that this study showed *some evidence of carcinogenic activity* of PCP in male F344 rats, based on increased incidences of mesothelioma and nasal squamous cell carcinoma in the stop-exposure study. Additionally, the tumors observed in the 1-year stop-exposure study did not regress when animals were examined 1 year after exposure stopped.

Table 4-17. Incidences of treatment-related tumors in male F344 rats fed purified PCP for up to 2 years

Tumors and statistical analysis	Dose (mg/kg-day)				
	0	10	20	30	60[a]
Malignant mesothelioma Overall rate[b] Adjusted rate[c]	1/50 (2%) 2.6%	0/50 (0%) 0%	2/50 (4%) 5.1%	0/50 (0%) (0%)	9/50 (18%) 20.6%
Statistical analysis Poly-3 test[d] Fisher's exact test	p = 0.447N	p = 0.509N p = 0.500N	p = 0.511 p = 0.500	p = 0.472N p = 0.500N	p = 0.014 p = 0.008
Historical control incidence (mean ± standard deviation)	40/1,354 (3.0 ± 2.3%), range = 0–8%				
Nasal squamous cell carcinoma Overall rate[b] Adjusted rate[c]	1/50 (2%) 2.7%	3/50 (6%) 8.1%	1/50 (2%) 2.6%	0/50 (0%) (0%)	5/50 (10%) 11.7%
Statistical analysis Poly-3 test[d] Fisher's exact test	p = 0.171N	p = 0.299 p = 0.309	p = 0.756N	p = 0.471N p = 500N	p = 0.128 p = 0.102
Historical control incidence (mean ± standard deviation)	5/1,314 (0.5 ± 1.0%); range = 0–4%				

[a]Stop-exposure study; rats received treated feed for 52 weeks and untreated feed until study termination at 2 years.
[b]Number of animals with tumors/number of animals examined.
[c]Poly-3 estimated incidence after adjustment for intercurrent mortality.
[d]Trend-test under control column (60 mg/kg-day group excluded); pair-wise comparison test under treatment group column. Poly-3 test accounts for intercurrent mortality; N refers to negative trend.

Source: NTP (1999).

Schwetz et al. (1978) conducted a 2-year study in 25 male and 25 female Sprague-Dawley rats maintained on diets containing EC-7 (90.4% purity) at concentrations delivering doses of 3, 10, or 30 mg/kg-day; males were fed the diets for 22 months and females for 24 months. Tumors typical of this strain of rat (i.e., pituitary, adrenal and thyroid glands, testes, and pancreas tumors in males and pituitary, thyroid, mammary glands, and uterus tumors in females) were noted in 41% of the male controls and 100% of the female controls. The treated animals exhibited tumors that were also observed in the control animals. There were no statistically significant increases in incidence of tumors noted in the treated animals when compared with the controls. Information concerning individual tumors was not included in the report.

Initiation/promotion studies. Umemura et al. (1999) examined the initiating and promoting activity of aPCP (98.6% purity) administered in the diet to 20 male B6C3F$_1$ mice/group. Diethylnitrosamine (DEN) was given as the initiator when the promoting activity of aPCP was assessed, and PB was administered as the promoter when the initiating activity of aPCP was assessed. Table 4-18 summarizes the treatment protocol and response of each group to

treatment. Three groups of mice received no treatment during the 13-week initiating phase but were administered a basal diet, 600 ppm aPCP, or 500 ppm PB during the 25 week promoting phase. DEN was administered in drinking water to four groups for 13 weeks at a concentration of 20 ppm followed by a 4-week rest period. Following the rest period, animals were treated with a basal diet, 500 ppm PB in drinking water, or 300 or 600 ppm aPCP in the diet for 25 weeks to assess promoting activity of aPCP. aPCP was administered at 1,200 ppm during the initiating phase followed by no treatment for 29 weeks. Two groups of mice received aPCP at concentrations of 600 or 1,200 ppm in the diet for 13 weeks, followed by 500 ppm of PB for 29 weeks (no rest period). The doses corresponding to dietary concentrations of 300, 600, and 1,200 ppm aPCP were estimated to be 54, 108, and 216 mg/kg-day, respectively.

Table 4-18. Hepatocellular tumors in B6C3F$_1$ mice in initiation/promotion studies

Treatment[a]		Incidences				Tumor multi-plicity
Initiation (13 weeks)	**Promotion (25 weeks)**	**Altered foci**	**Adenomas**	**Carcinomas**	**Adenoma/ carcinoma**	
Untreated	Basal diet	0/20	0/20	0/20	0/20	0
Untreated	aPCP (108 mg/kg-day)	1/19 (5%)	0/19	0/19	0/19	0
Untreated	PB (500 ppm)[b]	8/20 (40%)	0/20	0/20	0/20	0
DEN (20 ppm)	Basal diet	7/15 (47%)	4/15 (27%)	0/15	4/15 (27%)	0.33
DEN (20 ppm)	PB (500 ppm)	6/19 (32%)	10/19 (53%)	1/19 (5%)	10/19 (53%)	1.42[c]
DEN (20 ppm)	aPCP (54 mg/kg-day)	8/15 (53%)	10/15[c] (67%)	2/15 (13%)	10/15 (67%)[c]	1.27[c]
DEN (20 ppm)	aPCP (108 mg/kg-day)	13/18 (72%)	13/18 (72%)[d]	4/18 (22%)	13/18 (72%)[d]	2.22[c]
aPCP (216 mg/kg-day)	PB (500 ppm)[b]	5/20 (25%)	0/20	0/20	0/20	0
aPCP (216 mg/kg-day)	PB (500 ppm)[b]	2/20 (10%)	0/20	0/20	0/20	0/20
aPCP (216 mg/kg-day)	Untreated	2/17 (12%)	0/17	0/17	0/17	0/17

[a]Vehicle: aPCP in feed; DEN and PB in drinking water; a 4-week rest period followed the initiation phase.
[b]No rest period, PB given for 29 weeks.
[c]$p < 0.05$.
[d]$p < 0.01$ (compared with DEN + PB).

Source: Umemura et al. (1999).

Survival of mice was reduced in animals administered 108 (19/20) and 216 mg/kg-day (17/20) of aPCP alone. DEN-treated animals also exhibited a decrease in survival with basal diet (15/20), PB (19/20), and 54 (15/20), and 108 (18/20) mg/kg-day aPCP. Body weight measurements recorded at the end of the 42-week study showed significant reductions of 20, 22, 24, and 29% in mice receiving DEN followed by basal diet, PB, and 54, and 108 mg/kg-day aPCP, respectively, compared with mice receiving only the basal diet. Hepatomegaly was observed with aPCP or PB following DEN treatment. Liver weights were increased in mice receiving 108 mg/kg-day aPCP with (1.9-fold) or without (1.3-fold) prior DEN treatment. Liver weights in animals treated with PB alone (1.3-fold) or after aPCP treatment (1.4- and 1.3-fold with 108 and 216 mg/kg-day, respectively) were also increased. Liver weights were not increased after administering 216 mg/kg-day aPCP for 13 weeks, followed by no treatment for 29 weeks.

There was an increase in incidence of hepatocellular altered foci for all mice in the treated groups, although the only statistically significant increase (5.7-fold) in multiplicity was observed with DEN initiation and 108 mg/kg-day aPCP promotion. All groups initiated with DEN exhibited hepatocellular adenomas and carcinomas with the exception of the DEN control group, which only developed adenomas. The incidence of liver tumors was statistically significantly higher in mice initiated with DEN and promoted with 54 (67%) or 108 mg/kg-day PCP (72%) than in control mice receiving DEN only (27%). Tumor multiplicity was statistically significantly increased in 54 and 108 mg/kg-day aPCP-promoted mice (1.27 and 2.22 tumors/mouse, respectively) and 500 ppm PB (1.42 tumors/mouse) compared with DEN controls (0.33 tumors/mouse). No liver tumors developed in mice initiated with aPCP with or without subsequent promotion with PB. In this study, aPCP, at approximate doses of 54 and 108 mg/kg-day, showed promoting, but not initiating, activity in mice that were initiated with DEN. Umemura et al. (1999) concluded that aPCP exerts a promoting effect on liver carcinogenesis.

In another promotion study, Chang et al. (2003) administered an initiator, 100 µg dimethylbenzanthracene (DMBA) in acetone (100 µL), in a single application to the back of 10 CD-1 female mice/dose followed 1 week later by promotion treatment with 2.5, 50, or 1,000 µg PCP or TCHQ (purities not reported) in acetone twice weekly painted onto the skin of the mice for total treatment times of 20 or 25 weeks. DMBA treatment followed by PCP or TCHQ promotion resulted in a dose-related increase (≥1.6-fold) in epidermal hyperplasia and elevated proliferating cell nuclear antigen expression (≥2.2-fold), with TCHQ being slightly more effective than PCP. One or two skin tumors were observed in week 6 (30%) and week 11 (20%) in mice treated with PCP (0.2–0.4 tumors/mice average) and TCHQ (0.1–0.7 tumors/mouse average), respectively. Systemic effects include dose-related decreases in body weight in which TCHQ induced a greater loss in body weight than PCP (16 versus 7%, respectively). The kidneys were significantly enlarged for all treated mice. Liver and spleen weights were increased with PCP and decreased with TCHQ following treatment. However, PCP (not TCHQ)

promotion also caused lymphomas. Initiating ability of PCP or TCHQ was not tested in this study.

4.2.4.2. Inhalation Studies

No chronic cancer bioassays by the inhalation route of exposure are available.

4.3. REPRODUCTIVE, ENDOCRINE, AND DEVELOPMENTAL STUDIES

4.3.1. Reproductive and Endocrine Studies

Schwetz et al. (1978) conducted a one-generation reproductive toxicity study in which groups of 10 male and 20 female Sprague-Dawley rats were administered EC-7 (90% purity) in the diet. Dietary concentrations were adjusted monthly to deliver doses of 3 or 30 mg/kg-day. The test material was administered continuously for 62 days prior to mating and during mating, gestation, and lactation. All animals including pups were sacrificed after the litters were weaned on lactation day 21 (169 days for males; ~110 days for females). Toxic effects were noted in the animals and pups of the high dose only. There were no significant effects on survival, body weight, or litters at the low dose. Decreased body weight was noted in high-dose rats, with an 8% decrease in males and a 10% decrease (statistically significant) in females. At 30 mg/kg-day, fewer pups were born alive and the survival of pups decreased throughout lactation, leading to significantly decreased litter sizes measured on days 7, 14, and 21 of lactation. In addition, mean pup weights were significantly decreased by 14–27% at birth and throughout lactation at 30 mg/kg-day compared with the controls. Decreases in pup weight gain (28%) and survival (79%) during the first 14 days of lactation in the 30 mg/kg-day dose group are suggestive of a lactational effect of EC-7. The study authors noted that an increased incidence of litters with skeletal variations (lumbar spurs and vertebra with unfused centra) occurred at 30 mg/kg-day compared with controls. The study authors determined that the LOAEL for this study was 30 mg/kg-day for statistically significant changes in reproductive and developmental effects (decreased survival and growth, and skeletal variations); the NOAEL was 3 mg/kg-day.

In a two-generation reproductive toxicity study (Bernard et al., 2002), tPCP (88.9% purity) in corn oil was administered by gavage 7 days/week to groups of 30 male and 30 female Sprague-Dawley rats at doses of 10, 30, or 60 mg/kg-day. F0 male and female rats were given PCP for at least 70 days prior to mating and during mating, gestation, and lactation until weaning of litters, after which all F0 animals were sacrificed. F1 male and female rats were similarly exposed, starting at weaning and continuing through to the day before sacrifice. In addition to indices of reproductive performance, parameters of reproductive function (vaginal patency, preputial separation, estrous cycle, and sperm morphology) were also evaluated.

Absolute body weight of the 30 and 60 mg/kg-day groups of F0 and F1 parental male rats were statistically significantly decreased by 5.3 and 15%, respectively, compared with controls from day 36 throughout the remainder of the study. Significantly decreased absolute body weight was observed in 60 mg/kg-day females during the premating, gestation, and lactational

periods. No treatment-related effect was observed on body weight in females receiving 30 mg/kg-day, except for lactation days 10 and 15–17 in which body weight was statistically significantly lower (~8%) than controls. Systemic effects in parental animals (F0 and F1 male rats) were observed at 30 and 60 mg/kg-day dose levels and included increased liver weight, enlarged liver (F0 males only), and microscopic liver lesions ranging from centrilobular hypertrophy and vacuolation, multifocal inflammation, and single cell necrosis to a centrilobular pigment identified as LF. Centrilobular hypertrophy, vacuolation, and multifocal inflammation were also observed at the lowest dose of 10 mg/kg-day in F0 and F1 males. The liver weight in F0 females was significantly greater than controls in the 30 and 60 mg/kg-day dose groups. Parental females exhibited histopathological effects similar to males, including centrilobular hypertrophy and vacuolation, multifocal inflammation, single-cell necrosis (except for F1 females), and LF pigment at tPCP doses of 10, 30, and 60 mg/kg-day. Additionally, bile duct proliferation was observed at 60 mg/kg-day tPCP.

The fertility index and the number of litters produced were decreased at 60 mg/kg-day in F1 females. Days to vaginal patency and preputial separation were statistically significantly increased in F1 females (at doses ≥10 mg/kg-day) and males (at doses ≥30 mg/kg-day), respectively. The length of the estrous cycle was not significantly affected in either F0 or F1 females. Sperm morphology and count were not affected in F0 males, although testicular spermatid count and testes weight were decreased at 30 and 60 mg/kg-day in F1 males. Offspring evaluations showed significant reduction in mean litter size, number of live pups, viability index, and lactation index for F1 and/or F2 pups at 60 mg/kg-day tPCP compared with the controls. Body weight of pups was statistically significantly decreased by 6–9% at 10 mg/kg-day (lactation days 1-4), by 10–15% at 30 mg/kg-day (lactation days 1-28), and by 11–39% at 60 mg/kg-day (lactation days 1-28). In addition, decreased weights of the liver, brain, spleen, and thymus were observed in F2 pups at 60 mg/kg-day. The study authors determined that the parental LOAEL was 30 mg/kg-day for male and female rats based on significantly decreased body weight and weight gain in F1 generation parental rats, and testicular effects in F1 male rats (decreased testis weight, decreased spermatid count). The investigators noted that reproductive and developmental toxicity in the rats of this study were only observed at doses that also induced systemic toxicity. The EPA determined that the parental LOAEL was 10 mg/kg-day (lowest dose tested) for male and female parental rats, based on effects in the liver characterized by single cell necrosis, LF, centrolobular hypertrophy, cytoplasmic vacuolation, and multifocal inflammation. The parental NOAEL could not be determined. The reproductive LOAEL was 10 mg/kg-day (lowest dose tested) based on statistically significantly decreased group mean pup weight and statistically significantly increased vaginal patency in females. The reproductive NOAEL could not be determined.

Beard et al. (1997) conducted a study using mink to assess the effect of PCP in a one-generation study. Groups of 10 female mink (9 months old) received 1 mg/kg-day PCP (purity

not stated; recently confirmed as aPCP [CalEPA, 2006]) in the diet continuously for 3 weeks before and during mating, and throughout gestation and lactation of one litter of kits. Each female was mated twice with an untreated male mink, with an interval of 7–8 days between matings. Treatment with 1 mg/kg-day aPCP had no effect on clinical signs, body weight gain, or food consumption. No effect was observed on females accepting males during the first mating, but statistically significantly fewer aPCP-treated females accepted males during the second mating, resulting in significantly fewer pregnant females. Implantations were not affected by aPCP treatment, but only 70% of the treated mink with implantation sites eventually whelped compared with 88% of controls. In aPCP-treated mink, 46.7% of embryos were lost compared with 40.5% of control embryos, which resulted in smaller litter sizes (3.40 versus 4.45 for controls). The decreased implantation rate and reduced embryo survival after implantation were not statistically significantly different from the controls; however, the combined effect of these decreases contributed to the lower whelping rate. Uterine cysts were present in both control and treated mink, although the severity was greatest in the treated animals (severity grade 1.33 in treated versus 0.19 in controls). The study authors suggested that aPCP may have contributed to the increased loss of embryos. Beard et al. (1997) noted that the uterine cysts may have been associated with uterine infection and could indicate an immunosuppressive activity on the uterus by aPCP. Additionally, aPCP treatment resulted in a longer duration of pregnancy (4–5 days longer) compared with controls. aPCP treatment had no effect on serum levels of progesterone, estradiol, cortisol, or T_4 in adult female mink at weaning of their litters. Mink are seasonal breeding animals (in that ovulation is induced by copulation and implantation is delayed) which, according to the investigators, may result in these animals being particularly sensitive to aPCP (mild effects on reproduction were noted at a dose that was an order of magnitude lower than the NOAEL for a two-generation study in rats [Bernard et al., 2002]). A decrease (not considered statistically significantly different from controls) in the whelping rate was observed in mink at 1 mg/kg-day aPCP; however, it is unknown if this is a result of the embryo loss or the reduction in mating response. The study authors did not determine a NOAEL or LOAEL for this study. The EPA established a free-standing NOAEL of 1 mg/kg-day (only dose used), based on the absence of treatment-related toxicologically significant effects.

Beard and Rawlings (1998) examined reproduction in a two-generation study in mink exposed to 1 mg/kg-day PCP (purity not reported); 10 controls/generation were included. Dams (number of animals not reported) were administered PCP, in feed, 3 weeks prior to mating and continued through gestation until weaning of offspring (8 weeks postpartum). Eight F1 generation females (from treated dams) were administered PCP in their feed starting at weaning and animals were maintained on the treated diet as animals grew and were mated with untreated males. Treatment continued throughout gestation and lactation, and was terminated with sacrifice of F1 females 3 months after the end of the lactation period. Six F1 generation males were administered PCP in their feed starting at weaning until maximal development of the testis

(approximately 42 weeks of age), at which time the F1 males were sacrificed. Ten F2 generation females were administered PCP-treated feed from weaning until mink reached full body size (approximately 30 weeks of age). Eight F2 generation males were administered PCP-treated feed from weaning until the mink reached sexual maturity in their first breeding season. The study authors noted that all of the animals received PCP-treated feed continuously from conception to maturity. The only change observed in the body weights of PCP-treated mink was a 17% increase over controls in the body weight of F1 males. There were no changes in the proportion of F1 generation accepting the first and second mating. Additionally, no temporal changes were noted during the matings. PCP treatment did not affect whelping date or duration of gestation in the mink. Mean testis length was greater in PCP-treated F1 male mink compared with controls, although this difference was not apparent in examination (length and mass measurements) of testes after removal. Interstitial cell hyperplasia of greater severity was noted in the testes of F1 generation males compared with controls (severity scores for left and right testes were 1.0 and 0.6 for controls versus 2.3 and 2.5 for treated animals, respectively). The severity of cystic hyperplasia in the prostate gland of F1 males was statistically significant (0.9) compared with controls (0). A higher serum testosterone concentration was associated with the mild multifocal cystic hyperplasia, noted in 50% of the PCP-treated mink.

Serum T_4 secretion was statistically significantly decreased in the F1 (~21%) and F2 (~18%) males and F2 females (~17%). T_4 secretion was presented graphically in Beard and Rawlings (1998); therefore percent changes are reported as approximate values estimated from the graphs. Thyroid mass was decreased in both F1 and F2 generation animals, although the reduction was statistically significant only in F2 females (~27%). There was a significant increase in size (42%) of the adrenal gland in the F1 females, but no change in the F2 females. Interestingly, decreased mating and whelping rates were observed in mink treated with 1 mg/kg-day PCP in the one-generation study by Beard et al. (1997) compared with no changes in mating or whelping rates of 1 mg/kg-day PCP-treated mink in the two-generation reproductive study by Beard and Rawlings (1998). The authors noted that the treatment-related cystic hyperplasia of the prostate and interstitial hyperplasic testes may be associated with PCP-induced hypothyroidism. The study did not report a NOAEL or LOAEL. The EPA determined a LOAEL of 1 mg/kg-day based on significant decreases in T_4 secretion.

In a one-generation study, groups of 13 ewes (1–3 years old) received an untreated diet or a diet treated with PCP (purity not reported) at a concentration delivering a dose of 1 mg/kg-day (Beard et al., 1999a). The ewes were treated for 5 weeks prior to mating (with untreated rams), during gestation, and until 2 weeks after weaning their lambs. The ewes were sacrificed at the end of treatment. Clinical signs, blood hormone levels, ovarian function, embryonic growth, reproductive function, and histopathologic lesions were assessed during the study. No clinical signs or treatment-related decreases in body weight were observed. One ewe died of a cause unrelated to treatment with PCP. No effects on reproductive function (i.e., ovulation rate,

fertility rate, lambing rate, mean number of lambs born per ewe, and mean gestation rate) were observed. The male:female ratio showed an excess of ewe lambs born (5:13). There was a slight but statistically significant decrease in the weight of ewe lambs at weaning (86% of control weight). Ovarian function (follicle number and corpora lutea size), fetal growth (measured by head diameter), and post weaning serum levels of luteinizing hormone (LH), FSH, and cortisol were not affected by treatment with PCP. However, maximum serum T_4 levels in PCP-treated ewes were statistically significantly lower (approximately 25%) than in control ewes with or without prior administration of thyroid-stimulating hormone (TSH). The increase in serum T_4 levels compared with pretreatment level was 190% for PCP-treated ewes and 169% for controls.

Beard et al. (1999b) described a study in sheep in which the ram lambs born of ewes maintained on untreated or PCP-treated diets were examined. A dose of 1 mg/kg-day PCP (purity not reported) was administered starting at week 5 prior to mating and continuing through weaning of lambs. The lambs were maintained on the same diets as the ewes from weaning until puberty at 28 weeks of age. The lambs exhibited no overt signs of toxicity or treatment-related decreases in body weight. Testes diameter was unaffected at 10 and 14 weeks of age, but scrotal circumference measured at intervals between 16 and 26 weeks was statistically significantly increased in PCP-treated rams. There was no effect of PCP on age at puberty, sperm count, or sperm motility at 27 weeks of age. Scores for different measures of sexual behavior were consistently lower in PCP-treated rams than in controls at 26 weeks of age, but the differences were not statistically significant. T_4 levels were statistically significantly lower at 6–16 weeks, similar at 18–26 weeks, and lower at 28 weeks of age, compared with control levels. The response to TSH stimulation was unaffected by treatment with PCP. The serum levels of other endocrine hormones were unaffected by treatment with PCP. Microscopic examination of the testes and epididymides showed seminiferous tubular atrophy, reduced production of spermatocytes in the seminiferous tubules, and reduced density of sperm in the body of the epididymides but not in the head and tail of the epididymides. The investigators attributed the spermatogenic findings to the reduced thyroid hormone levels.

4.3.2. Developmental Studies

Larsen et al. (1975) reported on groups of 10 pregnant CD Sprague-Dawley rats administered 60 mg/kg aPCP (>99% purity) in olive oil by gavage on GDs 8, 9, 10, 11, 12, or 13 and maintained until GD 20. Controls received olive oil only. The percentages of resorptions ranged from 2.0 to 11.6% for controls and from 1.6 to 13.5% for treated dams. Additionally, the temperature of the treated animals increased significantly (increases ranged from 0.5 to 1.14°C) in animals treated on GDs 8, 9, or 10. The fetuses from dams receiving aPCP on GDs 8, 9, 10, or 12 weighed 12 to 20% less than those from controls; the weight of fetuses from dams treated on GD 11 or 13 were similar to those of controls. There was a small increase in the percentage of fetuses with malformations: 2% after treatment on GD 8 and 5.8% after treatment on GD 9.

No malformations were observed in control fetuses. The investigators attributed the fetal effects to maternal toxicity because a placental transfer experiment, performed concurrently with this study, indicated that only very small amounts (<0.1% of the administered dose/gram of tissue) of aPCP cross the placental barrier.

In a study conducted by Welsh et al. (1987), 20 Sprague-Dawley rats/sex/dose were administered diets containing aPCP (>99% purity) at dose levels of 60, 200, or 600 ppm (4, 13, or 43 mg/kg-day, respectively) for 181 days prior to mating. At the end of the 181-day dosing phase, male and female rats were mated for teratological evaluation. After mating, PCP administration in the diet continued through gestation until GD 20 when dams were sacrificed. Body weight gain in maternal rats exposed to aPCP was statistically significantly decreased at the high dose (76% of control). Food consumption was increased for all dose groups in the early part of gestation. Ringed eye (50%) and vaginal hemorrhaging (25%) were observed in dams of the 43 mg/kg-day dose group. The investigators suggested that the hemorrhaging was most likely related to the pregnancies. Pregnancy rates were low in all dose groups (77.5, 55, 84.2, and 85% for the 0, 4, 13, and 43 mg/kg-day dose groups, respectively); however, there was no effect on fertility. There were no dose-related effects on corpora lutea, implantation efficiency, or average number of implants/female. Decreased numbers of viable fetuses (due to early death) were observed at 43 mg/kg-day. Statistically significant increases in the percentage of females with two or more resorptions were observed at 13 and 43 mg/kg-day.

Dose-related decreases in fetal body weight were observed in males (10%) and females (8%) in the 13 mg/kg-day dose group and for males (36%) in the 43 mg/kg-day dose group. Analysis at the 43 mg/kg-day dose level was not complete due to an alteration in the sex ratio at this dose (100% male sex ratio at this dose was reported). Crown-rump lengths were decreased in a dose-related manner for males and females at doses ≥13 mg/kg-day. No significant alterations in external or sternebral observations were reported at any dose of aPCP in this study. An increased incidence of misshapen centra and an increase in fetal litters with at least two skeletal variations were observed at 13 mg/kg-day aPCP. The results of this study demonstrate toxicity of aPCP at 13 mg/kg-day in the form of increased percentage of female rats with two or more resorptions. However, this study is confounded by a lack of fetal data at the high dose and inconsistent and low percentages of pregnancy at each dose level of aPCP tested. The researchers suggest that PCP is embryotoxic and embryolethal rather than teratogenic. The EPA determined that the maternal LOAEL was 13 mg/kg-day, based on significantly increased resorptions, and the maternal NOAEL was 4 mg/kg-day. The developmental LOAEL was 13 mg/kg-day, based on dose-related increases in the incidence of skeletal variations and decreases in fetal body weight and crown-rump lengths. The developmental NOAEL was 4 mg/kg-day.

In a study conducted by Schwetz et al. (1974a), doses of 5.8, 15, 34.7, or 50 mg/kg-day tPCP (88.4% purity) or 5, 15, 30, or 50 mg/kg-day aPCP (>98% purity) prepared in corn oil were

administered by gavage to groups of pregnant Sprague-Dawley rats on GDs 6–15 (inclusive). The control group consisted of 33 rats. The numbers of animals in the 5.8, 15, 34.7, or 50 mg/kg-day tPCP dose groups were 18, 17, 19, and 15, respectively, and in the 5, 15, 30, and 50 mg/kg-day aPCP dose groups were 15, 18, 20, and 19 for the aPCP-treated rats, respectively. Additional groups of rats were administered 30 mg/kg-day aPCP and tPCP on GDs 8–11 or 12-15 of gestation. Maternal toxicity from aPCP was evidenced by decreased maternal weight gain at the 34.7 and 50 mg/kg-day tPCP and 30 and 50 mg/kg-day aPCP dose groups for GDs 6–21 (74% compared with control). For tPCP, weight gain was decreased 22 and 43% at the 34.7 and 50 mg/kg-day doses, respectively, when compared with controls. The dams were more affected by aPCP than tPCP. No other significant signs of maternal toxicity were observed.

The incidence of resorptions was increased at the three highest dose groups for both aPCP (statistically significant in the 30 and 50 mg/kg-day dose groups) and tPCP (statistically significant in all three dose groups). At the aPCP 50 mg/kg-day dose level, there were 100% resorptions; thus, no measurements were recorded for aPCP-treated animals at values >30 mg/kg-day. Resorptions were measured in 7, 9, 27, and 58% of fetuses and 56, 65, 95, and 93% of litters treated with 5.8, 15, 34.7, and 50 mg/kg-day tPCP, respectively. In animals treated with 5, 15, 30, and 50 mg/kg-day of aPCP, resorptions were found in 4, 6, 97, and 100% of fetuses and 5, 4, 100, and 100% of litters, respectively. Fetal body weight was statistically significantly decreased for aPCP at 30 mg/kg-day and for tPCP at 34.7 and 50 mg/kg-day, but actual values were not reported. The sex ratio showed a significant change from the controls with a predominance of male survivors in the 30 and 50 mg/kg-day doses of aPCP and 34.7 and 50 mg/kg-day doses of tPCP. Crown-rump length was decreased at 30 mg/kg-day aPCP (statistically significant) and 34.7 and 50 mg/kg-day tPCP. The litter incidence of soft tissue anomalies (subcutaneous edema) and skeletal anomalies (lumbar spurs and supernumerary lumbar, or fused ribs) was statistically significantly increased at 15, 34.7, and 50 mg/kg-day tPCP, but the data did not indicate a clear dose-response (i.e., the number of litters affected were greater at 34.7 than at 50 mg/kg-day). The litter incidence for similar soft tissue and skeletal anomalies was also statistically significantly increased at 15 and 30 mg/kg-day aPCP. The skeletal anomalies of the vertebrae and sternebrae occurred in a dose-related manner that was statistically significant at doses ≥30 mg/kg-day for both tPCP and aPCP. At the 5 mg/kg-day aPCP dose, the only significant effect observed was an increased number of fetal rats with delayed ossification of the skull (threefold increase over controls).

Rats were treated on GDs 8–11 or 12–15 with 34.7 mg/kg-day tPCP or 30 mg/kg-day aPCP to examine the effects on early or late organogenesis. Maternal body weight was significantly decreased following treatment with aPCP (67%) and tPCP (27%) on GDs 8–11. There were no dose-related decreases in maternal body weight in animals treated on GDs 12–15. Resorptions in the GD 8–11 treatment group were significantly increased in the aPCP and tPCP treated rats. Fetal body weight and crown-rump length were significantly decreased in animals

treated on GDs 8–11 with aPCP and tPCP. For the resorptions and changes in fetal body weight and crown-rump length, aPCP-treated animals exhibited more severe effects than those treated with tPCP, but both formulations showed significantly elevated levels of fetal resorptions when treated on GDs 8-11. On GDs 8-11, both aPCP and tPCP caused significant decreases in fetal body weight and crown-rump length at the 30 and 34.7 mg/kg-day doses, respectively, but only aPCP also significantly reduced these endpoints when administered on GDs 12–15. Incidence of subcutaneous edema was statistically significant in fetuses treated with aPCP (100%) and tPCP (82%) during GDs 8–11 and with aPCP (95%) during GDs 12–15. Skeletal anomalies of the ribs, vertebrae, and sternebrae were found in approximately 100% of the fetuses treated with aPCP or tPCP during GDs 8–11. The only skeletal effects observed during GDs 12–15 were significant increases in the incidence of delayed skull ossification (aPCP, 70%) and sternebrae anomalies (aPCP, 85%; tPCP, 82%). The authors postulated that this study was limited due to the increased resorptions and correspondingly reduced litter sizes at higher dose levels, but the results at lower doses were sufficient to indicate that the developing embryo is more susceptible to PCP during early organogenesis. The study authors identified the developmental NOAEL for tPCP as 5 mg/kg-day, which is equivalent to the adjusted dose of 5.8 mg/kg-day the authors used for this grade of PCP to account for impurities.

Based on the results of this study, aPCP was more toxic than tPCP in maternal and fetal rats. The EPA determined that the maternal LOAELs were 34.7 mg/kg-day for tPCP and 30 mg/kg-day for aPCP, based on significantly increased incidence of resorptions and decreased body weight; the maternal NOAEL was 15 mg/kg-day. The developmental endpoints differed according to the formulation of PCP used. The developmental LOAEL for aPCP was 5 mg/kg-day based on dose-related, significantly delayed ossification of the skull. The developmental NOAEL could not be established. The developmental LOAEL for tPCP was 15 mg/kg-day, based on dose-related, statistically significant increases in soft tissue and skeletal anomalies. The developmental NOAEL was 5.8 mg/kg-day.

Bernard and Hoberman (2001) observed effects in Crl:CD BR VAF/plus (Sprague-Dawley) rats administered tPCP (88.9% purity; >97.5% chlorinated phenols) that were similar to but less severe than those reported by Schwetz et al. (1974a). Groups of 25 pregnant rats were administered tPCP in corn oil via gavage at doses of 0, 10, 30, or 80 mg/kg-day on GDs 6–15 (inclusive). Animals were sacrificed for maternal and fetal examinations on GD 21. The mean maternal body weight gain was reduced by 15% at 80 mg/kg-day. Significant decreases in maternal food consumption at 80 mg/kg-day were 15 and 11% less than controls on GDs 6–9 and 9–12, respectively. Additionally, increased numbers of dams with resorptions (83 versus 41% for controls) were reported at 80 mg/kg-day.

Developmental toxicity was also observed at 80 mg/kg-day. Effects following tPCP administration included decreased litter size (86% of controls) and reduced fetal body weight (79% of controls). Litters from dams treated with 80 mg/kg-day had significantly increased

incidences of visceral (27 versus 5% for controls) and skeletal malformations/variations (96 versus 27% for controls). The visceral malformations included hydrocephaly, diaphragmatic hernia, and dilation of renal pelvis, while skeletal malformations were of the vertebral and sternebral type of anomalies. This study showed similar effects to those reported by Welsh et al. (1987) in Sprague-Dawley rats, but this particular strain may not be as sensitive to tPCP, or tPCP is not as toxic to the fetus as aPCP. The study authors determined that the maternal NOAEL for this study was 30 mg/kg-day and the maternal LOAEL was 80 mg/kg-day, based on increased incidence of resorptions and decreased maternal body weight gain. The developmental NOAEL was 30 mg/kg-day and the developmental LOAEL was 80 mg/kg-day, based on significantly increased visceral malformations and skeletal variations and decreased live litter size and fetal body weight.

Bernard et al. (2001) examined inseminated New Zealand white rabbits (20 rabbits/dose) administered tPCP (88.9% purity) by gavage at doses of 0, 7.5, 15, and 30 mg/kg-day on GDs 6–18 (inclusive). The dams were sacrificed for maternal and fetal examinations on GD 29. There was no dose-related maternal mortality or overt toxicity at any dose level. Decreases in maternal mean body weight were statistically significant for GDs 6–12 and 9–12 at 30 mg/kg-day. At this dose, body weight gain and food consumption showed overall decreases of 29 and 10%, respectively, when compared with controls. The decreases were too small to be considered statistically significant. The 15 mg/kg-day dose group showed a significant decrease in body weight gain for GDs 9–12 only.

The fetuses did not exhibit signs of mortality and developmental parameters were unaffected by the treatment. The researchers noted a dose-related reduction in implantations per doe that was consistent with a decrease in litter size, although these changes were not statistically significant. With one exception, there were no significant external, visceral, or skeletal malformations observed in the fetuses of treated does. In this study, treatment with tPCP up to 30 mg/kg-day did not result in developmental effects in rabbits. Since rabbits did not receive the 80 mg/kg-day dose that the rats in the Bernard and Hoberman (2001) study, it is not possible to compare the sensitivity of rabbits with that of the CD rat. The study authors determined that the maternal LOAEL was 15 mg/kg-day, based on significantly reduced body weight gain; the NOAEL was 7.5 mg/kg-day. The developmental LOAEL could not be established; the NOAEL was 30 mg/kg-day (the highest dose tested). The developmental and reproductive studies for PCP are summarized in Table 4-19.

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Table 4-19. Summary of NOAELs/LOAELs for developmental and reproductive studies for PCP

Species, strain	Dose (mg/kg-day)/ route/duration	Grade/type of PCP	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Reference
Mink (10 F/dose)	0 or 1 (feed) One generation	aPCP	1	ND	Beard et al., 1997[a]
Mink (8 F/dose)	0 or 1 (feed) Two generations	PCP[b]	1	ND	Beard and Rawlings, 1998[a]
Sheep (13 F/dose)	0 or 1 (feed) One generation	PCP[b]	1	ND	Beard et al., 1999a[a]
Sheep	0 or 1 (feed) Two generations	PCP[b]	ND	1	Beard et al., 1999b[a]
Rat, Sprague-Dawley (10 M and 20 F/dose)	0, 3, or 30 (feed) 110 days, one generation	EC-7	3	30	Schwetz et al., 1978
Rat, Sprague-Dawley (30/sex/dose)	0, 10, 30, or 60 (gavage) 110 days, two generations	tPCP	ND	10	Bernard et al., 2002[a]
Rat, Sprague Dawley (20/sex/dose)	0, 4, 13, or 43 (feed) 181 days	aPCP	4	13	Welsh et al., 1987[a]
Rat, Sprague-Dawley (15–20 pregnant dams/dose)	0, 5, 15, 30, or 50 (gavage) GD 6–15	aPCP	ND	5	Schwetz et al., 1974a[a]
	0, 5.8, 15, 34.7, or 50 (gavage) GD 6–15	tPCP	5.8	15	
Rat, Sprague-Dawley (10 pregnant dams/dose)	0 or 60 (gavage) GD 8, 9, 10, 11, 12, or 13−20	aPCP	ND	60	Larsen et al., 1975
Rat, Sprague-Dawley (15–20 pregnant dams/dose)	0, 10, 30, or 80 (gavage) GD 6–15	tPCP	30	80	Bernard and Hoberman, 2001
Rabbit, New Zealand (20 pregnant dams/dose)	0, 7.5, 15, or 30 (gavage) GD 6–18	tPCP	30	ND	Bernard et al., 2001

[a]NOAELs and LOAELs determined by EPA for these studies; values for both genders unless otherwise specified.
[b]Purity not reported.
ND = not determined.

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4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Oral

4.4.1.1 *Acute Studies*

The oral median lethal dose (LD_{50}) for male and female rats receiving tPCP (90.4%) by gavage was reported as 155 mg/kg for males and 137 mg/kg for females by Norris (1972). Deichmann et al. (1942) reported oral LD_{50} values of 27.3 mg/kg for rats administered PCP in 0.5% Stanolex fuel oil, 77.9 mg/kg for PCP administered in 1% olive oil, and 210.6 mg/kg for sodium pentachlorophenate administered in a 2% aqueous solution. Oral LD_{50} values for mice, rats, and hamsters ranged from 27 to 175 mg/kg as reported by the International Agency for Research on Cancer (IARC, 1999). Clinical signs observed in dogs, rabbits, rats, and guinea pigs consisted of increased blood pressure, hyperpyrexia, hyperglycemia, glucosuria, and hyperperistalsis; increased urinary output followed by decreased urinary output; and rapidly developing motor weakness. Dying animals showed signs of complete collapse, asphyxial convulsive movements, and rapid onset of rigor mortis upon death. Necropsy examinations showed vascular damage with heart failure, and involvement of parenchymous organs (Deichmann et al., 1942).

4.4.1.2. *Immunotoxicity Studies*

McConnachie and Zahalsky (1991) reported that 38 individuals exposed to PCP (in PCP-treated log homes) for various times ranging from 0 to 13 years had activated T-cells, autoimmunity, functional immunosuppression, and B-cell disregulation. In addition, females, but not males, exhibited statistically significantly increased natural killer cell function. The exposed individuals consisted of 17 females 9–60 years of age (mean: 30.1 years) and 21 males 8-60 years of age (mean: 31.8 years). The exposed group was compared with a control group consisting of 120 individuals; 81 females and 39 males ranging in age from 11 to 50 years and from 24 to 67 years, respectively. Blood serum PCP concentrations ranged from 0.01 to 3.40 ppm (blood serum of 17 individuals was not analyzed for PCP content).

Daniel et al. (1995) studied immune response using peripheral lymphocytes from 188 patients exposed to PCP-containing pesticides for more than 6 months. Of those tested, the mitogenic response was impaired in 65% of patients. The likelihood of an impaired response was greatest in patients with blood PCP levels >10 μg/L (68%) and particularly for those with levels >20 μg/L (71%). Only 50% of patients with blood levels <10 μg/L had impaired immune response. The impaired response persisted for up to 36 months in some patients. Patients with impaired mitogenic response were also likely to have significantly elevated (3.2-fold) interleukin-8 (IL-8) levels and increased proportion of peripheral monocytes (18%) compared with patients with normal responses. The study authors concluded that PCP-exposed patients had moderate to severe immune dysregulation involving T and B lymphocytes. They further

noted that immune dysfunction may explain chronic infection, chronic fatigue, and hormonal dysregulation seen in PCP-exposed patients.

Exon and Koller (1983) conducted a study in rats to examine the effects of aPCP (97% purity) on cell-mediated immunity, humoral immunity, and macrophage function. Groups of male and female Sprague-Dawley rats were administered 5, 50, or 500 ppm aPCP (estimated average dose of 0.4, 4, or 43 mg/kg-day for males and 0.5, 5, or 49 mg/kg-day for females) continuously in the diet from weaning until 3 weeks after parturition. Offspring were treated similarly to the parents and treatment continued until 13 weeks of age. Immune response of offspring showed significant depression at all doses for cell-mediated immunity measured by delayed-type hypersensitivity reaction and humoral immunity measured by antibody production to bovine serum albumin (BSA). However, a clear dose-response relationship was not seen for either endpoint. In contrast to the lack of effect of aPCP in adult rats, exposure of rat offspring from the time of conception to 13 weeks of age produced effects on both humoral and cell-mediated immunity. Macrophage function measured by the rats' ability to phagocytize sheep red blood cells (SRBCs) increased in a dose-related manner that was statistically significant at 4 and 43 mg/kg-day for males and 5 and 49 mg/kg-day for females. In addition, there was an increase in the number of macrophages harvested from the peritoneal exudate.

An NTP study (1989) conducted in $B6C3F_1$ mice assessed the immunotoxic effect of aPCP at 200, 500, or 1,500 ppm, DP-2 and EC-7 at 200, 600, or 1,200 ppm, and tPCP at 200, 600, or 1,800 ppm in the diet for 6 months. Immunotoxicity was determined by measuring hemagglutination titers and plaque-forming cells (PFCs) in response to SRBC immunization. Mice showed marked decreases of 89 and 57% in PFCs in spleen cells in animals treated with 200 and 600 ppm tPCP (38 and 301 mg/kg-day for males; 52 and 163 mg/kg-day for females) respectively, and 45, 56, and 85% with 200, 600, and 1,200 ppm DP-2 (40, 109, and 390 mg/kg-day for males; 49, 161, and 323 mg/kg-day for females), respectively. EC-7 and aPCP measurements of PFCs increased and decreased, respectively, relative to controls, although results were not dose related. The hemagglutination titers were decreased in mice exposed to tPCP and DP-2, similar to the PFC response but with less consistency. The investigators suggested that this may have been due to the lack of sensitivity of the test. No dose-related effects were observed in measurements of hemagglutination with EC-7 or aPCP exposure.

Kerkvliet et al. (1982a) assessed the humoral immune response in groups of random-bred Swiss-Webster female mice fed tPCP (86% purity) at concentrations of 50, 250, or 500 ppm (estimated doses are 10, 51, or 102 mg/kg-day, respectively) and in B6 female mice fed 50, 100, or 250 ppm (estimated doses are 10, 20, or 49 mg/kg-day, respectively) for 8 weeks. In a separate experiment, groups of Swiss-Webster female mice were fed 250 ppm (51 mg/kg-day) tPCP with serial sacrifice at 2-week intervals during an 8-week feeding and an 8-week recovery period to determine the time of onset and recovery from PCP-induced toxicity. In addition, groups of B6 female mice were fed 1,000 ppm (195 mg/kg-day) aPCP (>99% purity) for 8 weeks

to assess the effect on immune function of a dose of aPCP fourfold higher than the tPCP dose. The effect of tPCP on the primary and secondary splenic antibody response to T-dependent SRBCs in Swiss-Webster mice was measured using the hemolytic antibody isotope release (HAIR) assay. The direct effect of tPCP on B-cells in B6 mice was measured using the splenic hemolytic plaque assay and the serum antibody response to the T-independent antigen, 2,4-dinitrophenyl-aminoethylcarbamylmethyl-Ficoll (DNP-Ficoll).

tPCP caused a dose-dependent suppression of the primary and secondary T-dependent immune responses in Swiss-Webster mice and the T-independent immune response in B6 mice. The kinetics of the response, peak of the response, and/or the magnitude of the prepeak and post peak antibody response to SRBCs were affected by tPCP at all doses. The IgM response was more sensitive to tPCP exposure than the IgG response. The serial sacrifice study in Swiss-Webster mice showed that significant immunosuppression was evident after only 2 weeks of tPCP treatment and persisted for the 8-week treatment and recovery periods. In contrast to tPCP, aPCP at a fourfold higher dose had no effect on humoral immune response in mice.

Kerkvliet et al. (1982b) studied the effect of tPCP and aPCP on susceptibility of mice to tumor growth and viral infection by assessing the function of cytotoxic T-cells and phagocytic macrophages. Male B6 mice were administered aPCP (>99% purity) or tPCP (86% purity) in the diet at concentrations of 50 or 500 ppm (average estimated doses are 10 or 102 mg/kg-day) for 12 weeks before testing for immune competence. In vivo immunotoxicity tests included: (1) growth of transplanted syngeneic 3MC-induced sarcoma cells, (2) susceptibility to Moloney sarcoma virus (MSV) inoculation followed by challenge with MSV-transformed tumor cells (MSB), and (3) susceptibility to encephalomyocarditis virus (EMCV) infection.

Progressive tumor growth was not affected by aPCP; the incidence was 35% for controls and 31 and 40% for the 10 or 102 mg/kg-day dose groups, respectively. The incidence of progressive tumor growth in tPCP-treated animals was significantly increased to 67 and 82% at 10 or 102 mg/kg-day, respectively. After MSV inoculation, all animals developed primary tumors that regressed, although at a slower rate in mice treated with 102 mg/kg-day tPCP. The tumor reappeared in 55% of the 102 mg/kg-day tPCP mice and two additional mice developed secondary tumors after challenge with MSBs for a total incidence of 73%. Secondary tumors developed in only 19% of controls and 18% of aPCP-treated mice, while 45% of tPCP-treated mice (10 mg/kg-day) developed secondary tumors. Splenic tumors were observed in MSB-challenged animals administered 10 (22%) and 102 mg/kg-day (44%) aPCP and 10 mg/kg-day (50%) tPCP, but not in the remaining 102 mg/kg-day tPCP-treated animals. In contrast to increased tumor susceptibility, susceptibility to EMCV-induced mortality was not significantly affected by either aPCP or tPCP. Of particular interest is the observation that treated mice showed significant depression of T-lymphocyte cytolytic activity and enhancement of macrophage phagocytosis after tPCP treatment but not after aPCP treatment. It is possible that these immune effects could be the result of exposure to the dioxin-like contaminants present in

tPCP (and not present in aPCP). However, Exon and Koller (1983) reported significant increases in macrophage phagocytosis in aPCP-treated rats.

Kerkvliet et al. (1985a) conducted a study to examine the effect of tPCP on the humoral immune response. $B6C3F_1$ mice were administered 15, 30, 60, or 120 mg/kg tPCP (86% purity) by gavage 2 days before challenge with SRBCs. The peak splenic IgM antibody response was measured 5 days after the challenge. The 120 mg/kg dose was given in two 60 mg/kg fractions on 2 consecutive days because a single 120 mg/kg dose was lethal to about one-half of the group of 32 animals. A dose-related immunosuppressive effect was observed with a 50% response (ID_{50} = median inhibitory dose) relative to controls at 83 mg/kg. aPCP (99% purity) at the same doses had no effect on the IgM antibody response. The investigators tested three contaminant fractions from tPCP at doses equivalent to that of the tPCP ID_{50} dose and found that the chlorinated dioxin/furan fraction had a significant immunosuppressive effect, whereas chlorinated phenoxyphenol and the chlorinated diphenyl ether fractions were ineffective.

Additionally, a comparison was made regarding the immunosuppressive effect of dietary tPCP administered for 6 weeks to two strains of mice ($B6C3F_1$ and DBA/2) at 10 or 250 ppm (average doses estimated as 2 and 49 mg/kg-day, respectively). Following tPCP administration, $B6C3F_1$ mice exhibited a greater immunotoxic effect than DBA/2 mice. The antibody response was suppressed 28 and 75% at 2 and 49 mg/kg-day tPCP, respectively, in $B6C3F_1$ mice compared with no significant suppression and 45% in DBA/2 mice, respectively. The investigators attributed the difference in the two strains to Ah-receptor responsiveness in $B6C3F_1$ mice and Ah-receptor-nonresponsiveness in DBA/2 mice (Kerkvliet et al., 1985a).

In another study, Kerkvliet et al. (1985b) examined the sensitivity of T-cells, macrophages, and natural killer cells in naive and interferon-induced female C57BL/6J (B6) mice to tPCP (86% purity) administered in the diet at concentrations of 100, 250, or 500 ppm (estimated average doses are 20, 49, or 98 mg/kg-day, respectively) for 8 weeks. Immune function tests included T-cell (concanavalin A and phytohemagglutinin induced) and B-cell mitogenesis (lipopolysaccharide [LPS] induced), mixed lymphocyte response (proliferation and cytotoxicity), spontaneous and boosted natural killer cytotoxicity, and phagocytic activity of resident peritoneal macrophages (thioglycollate-induced and tumor activated). Body weight was not affected, but the relative liver weights were significantly increased at all doses. The only effect observed was the mixed lymphocyte proliferative response to allogeneic stimulation. However, there was no effect on the generation of cytotoxic effector cells (measured by response to P815 mastocytoma cells); the peak proliferative response of mixed lymphocyte cultures did not show a clear dose-response. The T- and B-cell mitogenic response, natural killer cell activity, macrophage phagocytic activity, and bone marrow cellularity were not affected by exposure to tPCP. The investigators attributed the differences (i.e., humoral immunity was affected by tPCP, but cellular immunity was not) in response of humoral and cell-mediated immunity to inhibitory effects of tPCP.

Holsapple et al. (1987) administered PCP by gavage to groups of eight female $B6C3F_1$ mice at doses of 10, 30, or 100 mg/kg-day tPCP (purity not reported) or 100 mg/kg-day EC-7 (purity not reported) for 14 consecutive days. Spleen cells were harvested, cultured, and exposed to three antigens (LPS, DNP-Ficoll, and SRBCs) on day 15. Neither tPCP nor EC-7 affected the antibody response in the splenic cells immunized in vitro to LPS, DNP-Ficoll, or SRBCs. In another experiment, animals were treated as described above, but on day 10 or 11 the mice were immunized with SRBCs and sacrificed on day 15. The response of IgM-producing spleen cells was decreased in a dose-related manner with tPCP; the lowest dose of 10 mg/kg-day resulted in statistically significant reductions of 44 and 31% on day 4 (peak response) and day 5, respectively, compared with the controls. The study authors did not determine LOAEL/NOAEL levels.

White and Anderson (1985) demonstrated that tPCP (90.4% purity) administered to $B6C3F_1$ mice by gavage for 14 days inhibited the functional activity of complement measured by the microtiter hemolytic assay. The classical complement, spontaneous autoactivation, and alternative pathways were inhibited at the high dose (100 mg/kg). At 10 and 30 mg/kg, tPCP resulted in inhibitory effects that were less pronounced than high-dose effects. Animals that returned to the control diet after the 14-day treatment period showed only a partial recovery by 30 days post exposure. Animals treated with 100 mg/kg of EC-7 (91.0% purity, which contains relatively fewer dibenzo-p-dioxin/dibenzofuran contaminants compared with tPCP), exhibited no effects on complement levels. The investigators concluded that a contaminant or contaminants were responsible for the effect on the complement system.

In a study on cattle, McConnell et al. (1980) administered groups of three yearling (10–14 months old) Holstein cattle 100% aPCP, 10% tPCP/aPCP mix, 35% tPCP/aPCP mix, or tPCP to determine the effect of the level of contaminants in PCP. Each treatment group was given 647 ppm as PCP in feed (20 mg/kg-day body weight) for 42 days and then 491 ppm (15 mg/kg-day body weight) for 118 days of the study (total treatment time = 160 days). A group of three yearlings served as controls. McConnell et al. (1980) reported that IgG2 levels decreased as the proportion of tPCP increased. The decrease in IgM levels did not show a dose-related trend. Lymphocyte proliferation was increased in calves treated with tPCP following Concanavalin A and pokeweed mitogen activation. The increase was both time- and dose-related. Proliferation was not enhanced with the administration of aPCP, possibly suggesting that the dioxin/furan contaminants within tPCP were responsible for the proliferation.

Two groups of four female Holstein-Friesian cattle received either a control diet or tPCP-treated (purity 85–90%) diet corresponding to a dose of 0.2 mg/kg-day for 75–84 days followed by 2.0 mg/kg-day for 56–62 days (Forsell et al., 1981). Immunologic parameters measured included peripheral T- and B-cell populations, serum IgG, IgA, and IgM levels, mitogen-induced lymphocyte blastogenesis, and antibody response to SRBCs. The investigators observed no treatment-related effect on immune function in lactating cattle fed tPCP for up to 146 days.

These results are in contrast to those reported by McConnell et al. (1980), although the doses used by McConnell et al. (1980) were 7–10 times greater than the highest dose used by Forsell et al. (1981).

4.4.1.3. *Thyroid Hormone Studies*

Jekat et al. (1994) conducted a study to examine the effect of aPCP and tPCP (purity not reported) on thyroid hormones in female Wistar rats maintained on a normal iodine diet (NID) or a low iodine diet (LID) and pretreated with propylthiouracil to exacerbate the thyroid deficiency. Each group of eight female rats was administered 3 mg/kg-day tPCP, 3 or 30 mg/kg-day aPCP, or the vehicle only (0.5% tylose solution). The test materials were administered by gavage, twice a day at 12-hour intervals, 7 days/week for 28 days. Iodine deficiency caused a 182% increase in thyroid weight and decreased levels of total and free serum T_4 and T_3 and thyroid gland T_4, and T_3, and a decrease in the T_4:T_3 ratio in the serum and thyroid gland.

Treatment with 3 mg/kg-day aPCP caused decreases in total and free serum T_4, T_4:T_3 ratio in serum, and serum TSH. Treatment with 3 mg/kg-day tPCP caused decreases in serum T_4, serum T_3, T_4, and T_3 in the thyroid, T_4:T_3 ratio in serum, and serum TSH. Except for serum TSH, aPCP caused greater decreases in thyroid measurements for iodine-deficient rats than in normal rats. Because TSH levels were not elevated in response to the reduced thyroid hormone levels, the investigators concluded that PCP interfered with thyroid hormone regulation at the hypothalamic and pituitary levels. They also stated that peripheral interference with thyroid hormone metabolism was suggested by the greater reduction in T_4 compared with T_3. The study authors concluded that the NOAEL for this study was 3 mg/kg-day.

In a study by Rawlings et al. (1998), mature ewes in age groups of 1, 1–2, and 3–4 years and older were given capsules directly into the rumen twice weekly for approximately 6 weeks. The capsules contained 2 mg/kg aPCP (99.9% purity) or were empty (control). Blood was collected for serum analysis of T_4, LH, FSH, estradiol, progesterone, cortisol, and insulin on day 36 of treatment. A marked decrease in serum T_4 levels was observed in mature ewes at 36 days. In addition to statistically significant decreased serum T_4 levels, aPCP-treated ewes had significantly increased serum insulin levels. However, no treatment-related changes were observed in cortisol, LH, FSH, estradiol, or progesterone levels. No clinical signs or treatment-related weight changes were observed during treatment. The only microscopic change observed was increased severity of intraepithelial cysts in both oviducts.

In a study on cattle, McConnell et al. (1980) administered groups of three yearling (10–14 months old) Holstein cattle 100% aPCP, 10% tPCP/aPCP mix, 35% tPCP/aPCP mix, or tPCP to determine the effect of the level of contaminants in PCP. Each treatment group was given 647 ppm as PCP in feed (20 mg/kg-day body weight) for 42 days and then 491 ppm (15 mg/kg-day) for 118 days of the study (total treatment time = 160 days). A group of three yearlings served as controls. Treatment with aPCP caused statistically significant decreases in serum T_4

(60–71% of control level) and T_3 levels (56–65% of control level). The effect on thyroid hormones is attributable to PCP and not the contaminants, because hormone levels were similar among all treated groups of various grades of PCP. The investigators noted that thyroid follicles were smaller and more numerous in animals receiving 100% tPCP; they did not describe the thyroid of animals receiving aPCP.

Hughes et al. (1985) fed tPCP (85−90% purity) or aPCP (99.02% purity) to 15 Holstein bull calves (7 days old) twice daily at doses of 0, 2, or 20 mg/kg-day. One calf in each of the high-dose groups fed aPCP or tPCP died after acute toxicity (elevated temperature, rapid respiration, severe diarrhea, acute purulent pneumonia). After 5 days, the doses of 2 and 20 mg/kg-day were lowered to 1 and 10 mg/kg-day, respectively, and treatment was continued for a total duration of 42 or 43 days. Thyroid hormone levels in serum were measured during the first 35 days of treatment. Serum T_3 levels were reduced by 58–69% after treatment with 10 mg/kg-day tPCP and 49–55% with 10 mg/kg-day of aPCP. Treatment with 1 mg/kg-day reduced serum T_3 levels 44–56% with tPCP and 22–27% with aPCP. Reductions of 37–58 and 25% were observed in the calves' serum T_4 levels following treatment with 1 mg/kg-day tPCP and aPCP, respectively. T_3 and T_4 responsiveness to the TRH challenge were not affected by treatment with either grade. Organ weights most notably affected by PCP treatment were thymus and spleen in calves treated with 10 mg/kg-day tPCP or aPCP. The thymus weight was reduced by 83% with tPCP and 54% with aPCP. Microscopic lesions consistent with thymus atrophy were observed in tPCP-treated calves. Spleen weights were reduced by 52% with 10 mg/kg-day tPCP and by 32% with 10 mg/kg-day aPCP. Squamous metaplasia was observed in the Meibomian gland of the eyelid of the three calves treated with 10 mg/kg-day tPCP, but in none of the calves treated with aPCP. The investigators attributed the above eye effects to contaminants in PCP and not to PCP itself.

Beard and Rawlings (1998) examined reproduction in a two-generation study in mink exposed to 1 mg/kg-day PCP (purity not reported); 10 controls/generation were included. Dams (number of animals not reported) were administered PCP in feed 3 weeks prior to mating and continued through gestation until weaning of offspring (8 weeks postpartum). Eight F1 generation females (from treated dams) were administered PCP in their feed starting at weaning and maintained on the treated diet as animals grew and were mated with untreated males. Treatment continued throughout gestation and lactation, and was terminated with sacrifice of F1 females 3 months after the end of the lactation period. Six F1 generation males were administered PCP in their feed starting at weaning until maximal development of the testis (approximately 42 weeks of age), at which time the F1 males were sacrificed. Ten F2 generation females were administered PCP-treated feed from weaning until mink reached full body size (approximately 30 weeks of age). Eight F2 generation males were administered PCP-treated feed from weaning until the mink reached sexual maturity in their first breeding season. The study authors noted that all of the animals received PCP-treated feed continuously from

conception to maturity. T_4 secretion was presented graphically in Beard and Rawlings (1998); therefore, percent changes are reported as approximate values estimated from the graphs. Observed treatment-related effects included a statistically significant decrease in serum T_4 secretion in the F1 (21%) and F2 (18%) males and F2 females (17%). Thyroid mass was decreased in both F1 and F2 generation animals, although reduction was statistically significant only in F2 females (27%).

In a one-generation study, groups of 13 ewes (1–3 years old) received an untreated diet or a diet treated with PCP (purity not reported) at a concentration delivering a dose of 1 mg/kg-day (Beard et al., 1999a). The ewes were treated for 5 weeks prior to mating (with untreated rams), during gestation, and until 2 weeks after weaning their lambs. The ewes were sacrificed at the end of treatment. Maximum serum T_4 levels in PCP-treated ewes were statistically significantly lower (approximately 25%) than in control ewes with or without prior administration of TSH. The decrease in serum T_4 levels was observed over time, decreasing as night progressed.

Beard et al. (1999b) described a study in sheep in which the ram lambs born of five ewes maintained on untreated or PCP-treated diets were examined. A dose of 1 mg/kg-day PCP (purity not reported) was administered starting at week 5 prior to mating and continuing through weaning of lambs. The lambs were maintained on the same diets as the ewes from weaning until puberty at 28 weeks of age. T_4 levels were statistically significantly lower than control levels from 6 to 16 weeks, similar from 18 to 26 weeks, and lower again at 28 weeks of age. The response to TSH stimulation was unaffected by treatment with PCP. The serum levels of other endocrine hormones were unaffected by treatment with PCP. Microscopic examination of the testes and epididymides showed seminiferous tubular atrophy, reduced production of spermatocytes in the seminiferous tubules, and reduced density of sperm in the body of the epididymides, but not in the head and tail of the epididymides. The investigators attributed the spermatogenic findings to the reduced thyroid hormone levels.

4.4.1.4. *Endocrine Disruption Studies*

Orton et al. (2009) analyzed several pesticides, including PCP, for their ability to act as agonists or antagonists in estrogenic and androgenic receptor-mediated activity in vitro and in vivo. In yeast estrogen and androgen screen assays, PCP showed no agonistic activity, but was the most potent compound tested in antiestrogenic and antiandrogenic effects, which were statistically significant at concentrations from 0.015 to 7.8 μM ($p < 0.004$) and 0.015 to 3.9 μM ($p < 0.02$), respectively. In an ovulation assay, the ovaries were removed from female Xenopus laevis and monitored for dissociation of ovulated oocytes and hormone levels by radioimmunoassay following in vitro exposure to PCP at concentrations of 0.00625, 0.0625, 0.625, 6.25, and 62.5 μg/L. At the two highest concentrations, PCP statistically significantly depressed estradiol (62.5 μg/L, $p < 0.001$; 6.25 μg/L, $p < 0.01$) and testosterone ($p < 0.001$); 62.5 μg/L also depressed progesterone levels ($p < 0.001$). These effects were concurrent with

statistically significantly decreased ovulation at the highest three concentrations (62.5 and 6.25 µg/L, $p < 0.001$; 0.625, $p < 0.01$).

In the same study, adult female Xenopus laevis were consistently exposed to low, environmentally relevant concentrations of 0.1 and 1 µg/L PCP for 6 days and monitored for hormone fluctuations and alterations in ovarian morphology and function (Orton et al., 2009). Measured plasma progesterone levels were slightly elevated in both dose groups compared to the controls, although these were not significant unless both dose groups were pooled and compared to the controls (ANOVA $p = 0.036$). Alternately, the progesterone and testosterone levels from cultured ovarian tissue were lower than controls, with the low dose group more affected than the high dose group, but this data was not reported. In addition, degenerative ovarian features and abnormal oocytes were observed at higher levels in the low dose (6 and 22%, respectively) and high dose (11 and 22%, respectively) groups compared to controls (0 and 10%, respectively), although these levels did not reach statistical significance.

4.4.1.5. *Neurotoxicity Studies*

4.4.1.5.1. *In vitro studies*. Igisu et al. (1993) demonstrated that acetylcholinesterase activity in human erythrocytes is inhibited by PCP at temperatures ranging from 13 to 37°C. Using isolated sciatic nerve-sartorius muscle preparations from toads, Montoya and Quevedo (1990) demonstrated a dose-dependent irreversible reduction of end plate potential at the neuromuscular junction using PCP (purity not reported) concentrations between 0.01 and 0.1 mM. Axonal conduction, using an in vitro preparation of toad sciatic nerve, was shown to be blocked (concentration- and time-dependent) irreversibly by PCP (Sigma chemical; purity not reported but likely aPCP in the ionized form) at concentrations ranging from 0.3 to 10 mM (Montoya et al., 1988). PCP may not have reached the site of action as effectively in the ionized form as it would have been expected to if it were in the nonionized form. PCP was more potent (approximately twofold) in causing axonal conduction block than procaine. The median effective dose (ED_{50}) for PCP was 1 mM. PCP was also able to cause a dose- and time-dependent irreversible ganglionic synaptic transmission block at concentrations ranging from 0.003 to 0.03 mM. PCP is believed to have an effect during depolarization due to interference with Ca^{++} influx (Montoya and Quevedo, 1990).

Folch et al. (2009) exposed primary rat cerebellar granule neurons (CGNs) to 0.1-1000 µM PCP in vitro for 16 hour incubations and measured cell viability, apoptosis, ROS generation, and transcriptional activity of selected genes relevant to PCP-induced toxicity. In cells exposed to 100−1000 µM PCP, a statistically significant and dose-dependent loss of cell viability and increases in apoptosis, nuclear condensation, and ROS production were observed. These effects were concomitant with a significant up-regulation of genes related to oxidative stress (catalase, glutathione-S-transferase A5, glutathione peroxidase-1, and superoxide dismutase-1), apoptosis (caspases 3 and 8, p53, and Bcl-2 associated death promoter), cell cycle control (cyclins D1, A,

and E; cyclin-dependent kinases 2 and 4; cyclin-dependent kinase inhibitor 2B), and DNA damage (phosphorylated p53).

4.4.1.5.2. ***In vivo studies*****.** Savolainen and Pekari (1979) studied the neurochemical effects of tPCP (86.1% purity, sodium salt and 2.4% TCP) and the body burden of chlorophenols on groups of 5 male Wistar rats administered tPCP in drinking water at a concentration of 20 mg/L for 3–14 weeks. One group was allowed to recover for 4 weeks (total study duration 18 weeks). tPCP and TCP levels in the liver and brain (PCP only) remained stable between 3 and 14 weeks, whereas the levels in perirenal fat continued to increase during the treatment time. tPCP and TCP levels in liver, brain (PCP only), and fat decreased during the 4-week recovery period. Neurochemical studies showed that acid proteinase or superoxide dismutase (SOD) activities in the right cerebral hemisphere were statistically significantly increased at 8 or 14 weeks, respectively. NADPH-diaphorase activity was statistically significantly decreased in the right hemisphere at 3 and 18 weeks. Glutathione peroxidase activity in the right hemisphere was not significantly affected. Glutathione levels and SOD activity were decreased (statistically significant) in glial cells at 7 and 12 weeks. Glutathione levels were not affected in neuronal cells and glutathione peroxidase activity was not affected in glial cells. The study authors concluded that treatment with tPCP caused transient biochemical effects in the rat brain and that the effects were associated with body burden of chlorophenols and possibly dibenzo-p-dioxin and dibenzofuran contaminants.

Villena et al. (1992) examined the microscopic lesions in nerves of rats receiving PCP (purity not reported) under different experimental conditions. This study also included an examination of lesions in kidney and liver. Groups (number not reported) of male Wistar rats were given drinking water containing PCP at concentrations of 0.3 mM for 60 days, 1.0 mM for 60 or 90 days, 3.0 mM for 120 days, or drinking water without added PCP. Sciatic nerves were examined by electron and light microscopy. No effects were seen in rats given 0.3 or 1.0 mM for 60 days. Exposure to 1.0 mM PCP for 90 days or 3.0 mM PCP for 120 days caused changes in approximately 10% of type A and B nerve fibers in the myelin sheath. The effect was more severe in animals receiving the highest dose. Visible damage to the sciatic nerve fibers was characterized by variable degrees of dissociation of the myelin sheath, including complete dissociation, profound invagination of the myelin, advanced degeneration of the neuroglial coat, and variable losses of neurotubule neurofilaments, and other axoplasmic components. The investigators did not state whether the animals were treated with free tPCP, aPCP, or sodium salts. This specific information is important, considering that PCP has relatively low solubility in water (80 mg/L) (Budavari et al., 1996), while the sodium salt is freely soluble in water. It was noted that interference with food intake (malnutrition) can impair myelin development in maturing animals, but the study did not investigate whether PCP caused effects on body weights, food or water consumption, or clinical signs in this study.

As part of its investigation into the carcinogenicity of PCP in mice, NTP (1989) also conducted studies in groups of 10 $B6C3F_1$ mice/sex/dose to assess the neurobehavioral effect of PCP. Estimated doses of tPCP (38 and 301 mg/kg-day for males and 52 and 163 mg/kg-day for females), DP-2 (40, 109, or 390 mg/kg-day for males and 49, 161, or 323 mg/kg-day for females), EC-7 (36, 124, or 282 mg/kg-day for males and 54, 165, or 374 mg/kg-day for females), or aPCP (102, 197, or 310 mg/kg-day for males and 51, 140, or 458 mg/kg-day for females) were administered in the diet for 6 months. Neurobehavioral effects were assessed at weeks 5 and 26. The battery of tests included the presence or absence of autonomic signs; pinnal, corneal, and righting reflexes; spontaneous motor activity; acoustical startle response; visual placement response; grip strength; and rotarod tests.

At week 5, the only neurobehavioral effects observed were dose-related decreases in motor activity and rotarod performance in mice administered tPCP. At week 26, dose-related increases in motor activity and startle response were observed in female mice administered all four grades of PCP, while this effect in males was only observed in those receiving tPCP. Actual incidence data were not published in the NTP report; therefore, the effect level is not known with certainty.

4.4.2. Inhalation

4.4.2.1. *Acute Studies*

Hoben et al. (1976b) conducted a study in which groups of 12 male Sprague-Dawley rats were exposed to PCP (purity not reported) aerosols by inhalation exposure. Assuming an inhalation rate of 80 mL/minute, rats received calculated PCP doses of 10.1 and 14.5 mg/kg following exposure durations of 28 and 44 minutes, respectively. The dose-response curve was very steep; 33% of animals receiving 10.1 mg/kg died and 83.3% receiving 14.5 mg/kg died. The LD_{50} was 11.7 mg/kg.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genetic Toxicity Studies

Genotoxicity studies following PCP exposure have shown that, while mutations have not been detected in prokaryotic systems, there is evidence both in subcellular systems and in human cells in vitro that PCP can induce damage to DNA and proteins via oxidative mechanisms. In addition, gene mutation and recombination in fungi, clastogenic effects in mammalian systems in vitro, and a weakly positive indication of transplacental mutation in mice have been have been observed in assays with PCP. TCpHQ, a metabolite of PCP, has also been shown to induce DNA damage in in vitro studies and oxidative damage in both in vitro and in vivo studies.

4.5.1.1. *In Vitro Studies*

Exposure to tPCP (90.6 purity) at concentrations of 0.3, 1, 3, 10, or 30 μg/plate for 20 minutes did not induce mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, or TA1537 with or without the microsomal fraction (S9) from Aroclor 1254-induced rat or hamster liver (Haworth et al., 1983). Waters et al. (1982) reported PCP, at concentrations up to 10 μg/plate, was negative for mutations in *S. typhimurium* (tester strains TA98, TA100, TA1535, TA1537, and TA1538) in the presence and absence of S9. Donnelly et al. (1998) reported no increases in mutations in *S. typhimurium* (tester strains TA97a, TA98, and TA100) incubated with aPCP (>98% purity) at concentrations 2, 20, 50, 100, or 200 μg/plate. Buselmaier et al. (1973) reported that PCP was negative for mutations in *S. typhimurium* in the presence of S9. Gopalaswamy and Nair (1992) incubated 50 or 100 μg/plate PCP with *S. typhimurium* tester strain TA98, with and without S9. The changes relative to control could not be calculated; however, the authors reported a positive response in the number of revertants per plate (albeit a weak response) with both doses of PCP in the presence of S9 only.

Fahrig (1974) incubated 0.19 mM PCP with *Saccharomyces cerevisiae* for 6 hours to measure the mitotic gene conversion at the *ade2* and *trp5* loci. The number of convertants per 105 survivors was measured as a 15- and 12-fold increase over control at the *ade2* and *trp5* loci, respectively. The survival was reported as 30%.

Jansson and Jansson (1986) reported that forward mutations (6-thioguanine resistance [TGr]) were not induced in V79 Chinese hamster cells incubated for 24 hours with 6.25–50 μg/mL PCP (>99.5% purity). Cell survival was reduced (100, 90, 73, 53, and 27% cell survival) with increasing doses (0, 6.5, 12.5, 25, and 50 μg/mL, respectively). The authors concluded that the dose-dependent decrease in survival was possibly a result of PCP-induced inhibition of oxidative phosphorylation.

Jansson and Jansson (1991) examined the effects of two PCP metabolites, TCpHQ (doses of 4, 20, 40, and 60 μM) and TCpCAT (TCC; doses of 15, 30, 60, and 120 μM), on TGr at the hypoxanthine phosphoribosyltransferase (HPRT) locus and ouabain resistance (OuaR) at the Na/K-ATPase locus in V79 Chinese hamster cells in the absence of exogenous activation. The study demonstrated that the metabolite, TCpHQ, induced TGr at concentrations ≥20 μM. However, TCC did not induce TGr at any of the administered doses. Neither TCHQ nor TCC affected the frequency of OuaR mutants. The authors suggested that autoxidation of TCHQ to form the semiquinone radical or reactive oxygen species (ROS) would result in DNA damage (Jansson and Jansson, 1991).

Jansson and Jansson (1992) investigated the induction of micronuclei in V79 Chinese hamster cells treated with 5, 10, 15, or 20 μM TCHQ (>99% purity) for 3 hours. The survival of the V79 cells was significantly reduced following administration of TCHQ, and a LD_{50} of 12 μM was identified. Cells with micronuclei (per 2,000 cells scored) were significantly increased at

doses of ≥10 μM (increased threefold or more over controls) and was dose-dependent. The 5 μM dose induced micronuclei, but the increase was not considered statistically significant.

Galloway et al. (1987) assayed chromosomal aberrations (CAs) in Chinese hamster ovary (CHO) cells treated with 3, 10, 30, or 100 μg/mL with S9 and 10, 30, or 100 μg/mL without S9. tPCP produced a weakly positive response with added S9 at concentrations of 80 and 100 μg/mL; the response was negative without S9. Fahrig (1974) reported a weakly positive CA response with PCP in human lymphocytes in the absence of S9.

Galloway et al. (1987) investigated the effects of 1, 3, 10, or 30 μg/mL tPCP (91.6% purity) in the presence and absence of S9 in CHO cells. Weakly positive results were observed in the induction of sister chromatid exchanges (SCEs) in the absence of S9. The relative changes in SCEs per chromosome in treated versus control cells were 98.8, 120.5, 108.4, and 113.3% for 1, 3, 10, and 30 μg/mL, respectively. All but the lowest dose exhibited changes that were statistically significant. A negative response was observed in the CHO cells treated with tPCP in the presence of the S9 fraction.

Ehrlich (1990) showed that PCP (purity not reported) at 5, 10, or 20 μg/mL was not effective in inducing single strand breaks (SSBs) in CHO cells, whereas its metabolite, TCpHQ, was very effective. At a concentration of 10 μg/mL, PCP failed to induce SSBs after incubating with CHO cells for 2 hours; this concentration was only slightly toxic to cells after 3 days. After incubation for 2 days at a concentration of 20 μg/mL, PCP stopped growth of CHO cells. At concentrations of 2, 5, and 10 μg/mL, TCpHQ caused a dose-related increase in SSBs. Toxicity tests showed that 5 μg/mL of TCpHQ inhibited growth of CHO cells, 10 μg/mL stopped growth, and 20 μg/mL was toxic and killed the cells. Carstens et al. (1990) also found SSBs with TCHQ exposure when they administered 50 μM TCHQ to PM2 DNA. Within 1 hour of incubation, 0.58 SSB per PM2 DNA molecule were observed.

Dahlhaus et al. (1995) combined Chinese hamster V79 lung fibroblasts with 6.25, 12.5, 25, or 50 μM TCpHQ for 1 hour. There was no change in SSBs at doses ≤12.5 μM; however, SSBs increases were statistically significant at the 25 and 50 μM doses, compared with control. As cytotoxicity can induce SSBs, Dahlhaus et al. (1995) also examined the cytotoxic effects of TCpHQ. The cytotoxicity at 25 μM was statistically significant, but low, and did not parallel the SSBs. At 50 μM the cytotoxicity was much greater and corresponded with an increase in SSBs. The authors suggested that the toxic effects to the cells may also result in SSBs in DNA. In another study, Dahlhaus et al. (1996) found that 25 μM TCpHQ or TCpBQ incubated with Chinese hamster V79 cells significantly induced DNA fragmentation while TCoHQ, TCoBQ, and PCP did not.

Lin et al. (2001a) examined the effects of DNA fragmentation using TCpHQ and TCpBQ in the presence of the reducing agent NADPH and Cu(II), which have been shown to induce redox cycling in quinones. Calf thymus DNA treated with either TCpHQ (100 μM and 1 mM) and 100 μM Cu(II) or TCpBQ (1 and 10 μM) and 100 μM Cu(II) and NADPH caused an

increase in SSBs that was dose-dependent. TCpBQ alone (TCpHQ was not analyzed alone) did not induce SSBs.

Epithelial cells were isolated by Tisch et al. (2005) from human nasal tissue removed in the surgical treatment of chronic sinusitis and nasal concha hyperplasia. Cultures were exposed to aPCP (0.3, 0.75, and 1.2 mmol) for 1 hour and then examined for single and double strand breaks. DNA migration length was measured in treated cells and migration exceeding 35 µm was considered indicative of cell damage. There was an increase in the damaged cells observed in the middle nasal concha with 0.3 (1.4-fold), 0.75 (2.2-fold), and 1.2 mmol/mL (2.8-fold) PCP compared with the control. Similarly, the inferior nasal concha exhibited 1.2-, 1.7-, and 2.3-fold increases in damaged cells compared to the control following administration of 0.3, 0.75, and 1.2 mmol/mL PCP, respectively. Cells from both the inferior and middle (location of most of the wood dust-induced adenocarcinomas of the nose) nasal conchae were found to have severely fragmented DNA, observed with clear dose dependence. DNA damage in the middle nasal concha was observed in more than 50, 70, and 92% of PCP-treated cells. The inferior nasal concha exhibited less sensitive effects, with only 64% of treated cells showing DNA damage at the high dose (1.2 mmol/mL). While supportive of other in vitro testing, it should be noted that this ex vivo work used cells lacking the protective mucosal barrier present in vivo.

Purschke et al. (2002) used normal human fibroblasts to assess DNA damage via comet assay and DNA repair via unscheduled DNA synthesis (UDS) resulting from exposure to TCHQ or TCBQ at concentrations up to 60 µM. These experiments were designed to establish whether TCHQ or its metabolic by-product, H_2O_2, caused DNA damage. There were dose-dependent increases in DNA breakage with concentrations >20 µM H_2O_2 and ≥5 µM TCHQ, indicating that TCHQ caused DNA damage similar to H_2O_2, although at lower concentrations. TCHQ was far more potent than H_2O_2 in inducing DNA damage at concentrations between 0.5 and 10 µM, while TCBQ was less potent than H_2O_2. DNA damage produced by TCHQ, as measured by the relative tail moment, was still measurable at 24 hours after exposure, while damage produced by H_2O_2 had disappeared after 6 hours. In the UDS test, TCHQ-induced [^{3}H]thymidine incorporation peaked at 10 µM but fell to near-control levels at 25 µM, while H_2O_2-induced UDS continued to rise linearly up to at least 60 µM, indicating that TCHQ inhibited repair of the DNA damage it induced, while H_2O_2 did not. The fact that TCBQ, the autoxidation product of TCHQ, did not display the same genotoxic potency as TCHQ, was seen as evidence that redox cycling was not involved in the observed effects. The authors suggested that the tetrachlorosemiquinone radical may be responsible for any genotoxic activity of TCHQ.

Additionally, Purschke et al. (2002) exposed human fibroblasts to TCHQ to discern whether the semiquinone or the hydroxyl radical formed during redox cycling was responsible for the DNA damage by comparing TCHQ with H_2O_2. Based on kinetics of [^{3}H]thymidine incorporation, the authors suggested that DNA repair may be different following TCHQ exposure, as compared to H_2O_2 exposure. Mutagenicity of TCHQ, shown previously by Jansson

and Jansson (1991) at cytotoxic concentrations, was confirmed here at nontoxic concentrations; H_2O_2 did not induce mutants at concentrations 5 times higher than those needed for DNA damage (up to 50 µM). However, TCHQ mutation frequency (as measured in V79 cells with the HPRT assay) was significantly increased at 5 and 7 µM. These results confirmed the ability of TCHQ to induce mutations and that the effect was not caused by the metabolic by-product H_2O_2. The study indicates that in blocking DNA repair, TCHQ exposure permits sustained DNA damage that could lead to mutations.

Synopses of findings from genotoxicity studies with PCP are provided in Table 4-20, and results of genotoxicity studies with PCP metabolites are provided in Table 4-21.

Table 4-20. Summary of selected in vitro genotoxicity studies of PCP

Test System	Result (S9)	Reference
Reverse mutation in *S. typhimurium*	Negative (+/–)	Haworth et al. (1983)
Reverse mutation in *S. typhimurium*	Negative (+)	Gopalaswamy and Nair (1992)
Forward mutation (TGr) in V79 Chinese hamster cells at the HPRT locus	Negative (–)	Jansson and Jansson (1986)
DNA damage in *Bacillus subtilis*	Positive	Waters et al. (1982)
DNA damage in *S. cerevisiae* D3	Positive	Waters et al. (1982)
DNA damage in *S. cerevisiae* MP-1	Positive (–)	Fahrig (1978)
DNA damage in polA$^-$ *Escherichia coli*	Negative	Waters et al. (1982)
SSBs in V79 Chinese hamster cells	Negative (–)	Dahlhaus et al. (1996)
SSBs in CHO cells	Negative (–)	Ehrlich (1990)
SSBs in mouse embryonic fibroblasts	Weakly positive (+)	Wang and Lin (1995)
Single and double strand breaks in human mucosal cells	Positive (–)	Tisch et al. (2005)
CAs in CHO cells	Negative (–)	Galloway et al. (1987)
	Weakly positive (+)	Galloway et al. (1987)
CAs in human lymphocytes	Weakly positive (–)	Fahrig (1974)
SCE in CHO cells	Negative (–)	Galloway et al. (1987)
	Weakly positive (+)	Galloway et al. (1987)

Table 4-21. Summary of selected in vitro genotoxicity studies of metabolites of PCP

Test System	Result (S9)	Reference
TCpHQ		
Forward mutation (TGr) in V79 Chinese hamster cells at the HPRT locus	Positive (–)	Jansson and Jansson (1991)
Forward mutation (OuaR) in V79 Chinese hamster cells at the HPRT locus	Negative (–)	Jansson and Jansson (1991)
Forward mutation in V79 Chinese hamster cells at the HPRT locus	Positive	Purschke et al. (2002)
SSBs in V79 Chinese hamster cells	Positive (–)	Dahlhaus et al. (1996, 1995)
SSBs in CHO cells	Positive (–)	Ehrlich (1990)
SSBs in human fibroblasts	Positive	Carstens et al. (1990)
SSBs in calf thymus DNA	Positive	Lin et al. (2001a)
Strand breaks in human fibroblasts	Positive	Purschke et al. (2002)
TCoHQ		
SSBs in V79 Chinese hamster cells	Negative (–)	Dahlhaus et al. (1996)
TCpBQ		
SSBs in V79 Chinese hamster cells	Positive (–)	Dahlhaus et al. (1996)
SSBs in calf thymus DNA	Positive	Lin et al. (2001a)
TCpCAT[a]		
Forward mutation (TGr) in V79 Chinese hamster cells at the HPRT locus	Negative (–)	Jansson and Jansson (1991)
Forward mutation (OuaR) in V79 Chinese hamster cells at the HPRT locus	Negative (–)	Jansson and Jansson (1991)

[a]TCpCAT = Tetrachlorocatechol.

4.5.1.2. *In Vivo Studies*

A bone marrow micronucleus test was conducted using male and female CD-1 mice dosed by gavage with 24, 60, or 120 mg/kg tPCP (88.9% purity) for males and 10, 50, or 100 mg/kg tPCP for females; tPCP produced no increases in the frequency of micronuclei in this study conducted with male and female CD-1 mice (Xu, 1996).

In a bone marrow micronucleus test, male F344/N rats (five animals/dose) were treated i.p. with 25, 50, or 75 mg/kg PCP 3 times at intervals of 24 hours (NTP, 1999). Similarly, male $B6C3F_1$ mice were treated with 50, 100, or 150 mg/kg PCP. Neither the rats nor the mice showed an increase in micronucleated polychromatic erythrocytes (PCE) at any dose of PCP. The high dose was lethal in the rats (75 mg/kg) and the mice (150 mg/kg).

Daimon et al. (1997) conducted an in vivo/in vitro study that showed PCP (purity not reported) induced a significant increase in SCEs in hepatocytes isolated from male F344 rats injected i.p. with 10 mg/kg PCP. This was not accompanied by an increase in replicative DNA

synthesis, indicating that cell proliferation was not a factor in SCE induction. Chromosomal aberrations, however, were not observed in these cells.

Spalding et al. (2000) used nine chemicals, among them PCP (purity not stated), in two different transgenic mouse models: the heterozygous p53 knockout (p53+/–) mouse that is able to discriminate between genotoxic carcinogens and noncarcinogens and the v-Ha-ras gene (Tg·AC) transgenic mouse that can differentiate between genotoxic and nongenotoxic carcinogens and noncarcinogens. The findings were compared with results from standard 2-year bioassays conducted by NTP. PCP was administered to p53+/– mice for 26 weeks at 100, 200, or 400 ppm in the feed (estimated doses are 18, 35, or 70 mg/kg-day, respectively) and to Tg·AC mice via skin painting 5 days/week for 20 weeks at 30, 60, or 120 mg/kg-day. All doses used in this study were based on maximum tolerated doses (MTDs) from the corresponding 2-year bioassays. The highest dose of PCP in the feed, 400 ppm, caused signs of liver toxicity in the p53+/– mice, indicating that the MTD had been reached, but did not induce any tumors. In the Tg·AC mice, however, PCP induced papillomas in a dose-dependent fashion, with time-to-tumor decreasing with increasing dose, and tumor multiplicity increasing with dose. PCP induced some mortality in this study, but it showed inverse dose dependence (i.e., the highest mortality [38.5%] was observed at the lowest dose).

Yin et al. (2006) exposed 10 adult zebrafish/dose to 0.5, 5.0, or 50 μg/L aPCP (>98% purity) for 10 days to examine point mutations in the p53 gene. The number of mutated molecules measured in amplified liver cells of the zebrafish was significantly increased in the 5 and 50 μg/L dose groups compared with the control plasmid. The mutation rates were 7.33×10^{-4} and 10.73×10^{-4} at 5 and 50 μg/L aPCP, respectively. These mutation rates were more than threefold greater than those in control. The authors suggested that the induction of point mutations in p53 at concentrations as low as 5 μg/L aPCP may play a role in the carcinogenesis of PCP.

Peripheral lymphocytes of 22 male workers engaged in the manufacture of PCP (8 workers) or sodium-PCP (14 workers) were analyzed for chromosome aberrations; all 22 workers were smokers (Bauchinger et al., 1982; Schmid et al., 1982). Airborne PCP concentrations during the 3 years before the analysis showed 18/67 measurements <0.01 mg/m^3 and 10/67 measurements >0.5 mg/m^3 for the PCP workplace and 7/55 measurements <0.1 mg/m^3, and 8/55 measurements >0.5 mg/m^3 for the sodium-PCP workplace. The results for the workers exposed to PCP were compared with a group of 22 controls matched for age and social environment; 9 were smokers and 13 nonsmokers. The frequency of chromosome type aberrations (dicentrics and acentrics) was increased in PCP-exposed workers compared with the controls. The frequency of chromatid type aberrations (breaks and exchanges) was not statistically significantly increased compared with controls. A comparison of the SCE frequency in PCP workers who were all smokers with that of control smokers and control nonsmoker

subgroups showed that the SCE frequency could be attributed to smoking and not to PCP exposure.

Ziemsen et al. (1987) studied the frequency of SCEs and CAs in the lymphocytes of 20 adult workers occupationally exposed to airborne PCP at concentrations ranging from 1.2 to 180 μg/m^3 for 3–34 years. Fourteen workers were smokers and six were nonsmokers. Some workers were exposed via inhalation to dry PCP (96% pure) dust, technical water-soluble sodium-PCP (85% pure), or finished PCP solutions. Blood PCP concentrations ranged from 23 to 775 μg/L serum. No exposure-related effect was observed on the frequency of SCEs or chromosome aberrations in these 20 workers.

Table 4-22 presents a synopsis of the result from selected in vivo genotoxicity studies with PCP.

Table 4-22. Summary of selected in vivo genotoxicity studies of PCP

Test system	Result	Reference
Micronucleus formation in mice	Negative	NTP (1999); Xu (1996)
Micronucleus formation in rats	Negative	NTP (1999)
Sex-linked recessive lethal mutation in *Drosophila melanogaster*	Negative	Vogel and Chandler (1974)
Point mutations in p53 gene in hepatocytes of zebrafish	Positive	Yin et al. (2006)
Tumor multiplicity in v-Ha-ras transgenic mice TG·AC)	Positive	Spalding et al. (2000)
CAs in human lymphocytes	Weakly positive	Bauchinger et al. (1982)
CAs in human lymphocytes	Negative	Ziemsen et al. (1987)
CAs in male rat hepatocytes	Negative	Daimon et al. (1997)
SCE in human lymphocytes	Negative	Bauchinger et al. (1982)
SCE in human lymphocytes	Negative	Ziemsen et al. (1987)
SCE in male rat hepatocytes	Weakly positive	Daimon et al. (1997)

4.5.2. DNA Adduct Formation

4.5.2.1. *In Vitro Studies*

Lin et al. (2001a) incubated two PCP metabolites, TCpHQ and TCpBQ, at concentrations of 1 or 5 mM with 500 μg calf thymus DNA for 2 hours. TCpBQ induced the formation of four major adducts in a dose-dependent fashion. Estimated relative adduct levels (RALs) were 3.5 ± 0.93 per 10^5 total nucleotides at the high dose (5 mM). There were no adducts visible with controls. The authors reported (data not provided) that 1 mM TCpHQ [with and without Cu(II)] induced a pattern of DNA adducts similar to those induced by TCpBQ with an estimated RAL of 5.3 ± 0.1.8 per 10^7 total nucleotides.

Additionally, Lin et al. (2001a) attempted to induce depurination of these DNA adducts using thermal hydrolysis. The stability of the four major adducts following thermal hydrolysis indicated that apurinic (AP)/apyrimidinic sites observed with TCpBQ were not formed from depurination/depyrimidination of the adducts.

Dai et al. (2003) incubated deoxynucleosides (2 mM) in the presence of PCP (100 µM), H_2O_2 (100 µM and 1 mM), and myeloperoxidase and horseradish peroxidase (HRP). They found formation of an adduct between the oxygen of PCP and C8 of deoxyguanosine, but not with the three other deoxynucleosides. The reaction was specific for HRP, which is known to oxidize PCP to the phenoxy radical. However, when these researchers used rat liver microsome preparations with an NADPH-regenerating system and the same concentrations of PCP and nucleoside as above, a different adduct was formed, derived from TCpBQ. The results suggest that under in vivo conditions, PCP is likely to undergo two dechlorination steps before a DNA adduct can be formed. In a subsequent paper, Dai et al. (2005) presented evidence that p-benzoquinone derivatives can react with the amino and imino groups in the pyrimidine portion of the guanosine molecule to form a tricyclic benzetheno adduct.

4.5.2.2. *In Vivo Studies*

Lin et al. (2002) administered PCP (purity not reported, although likely aPCP as authors compared results to NTP [1999], which used aPCP, and earlier studies by Lin et al. [1999, 1997] used aPCP) to groups of three or four male F344 rats at concentrations of 30, 60, or 120 mg/kg-day for 1 day and concentrations of 30 or 60 mg/kg-day for 5 days and also obtained tissues from the livers of 10 F344 rats fed 60 mg/kg-day aPCP for 27 weeks in a 2-year bioassay conducted by NTP (1999). While no adducts were observed in the 1- or 5-day experiments, two adducts were identified in liver DNA in rats treated for 27 weeks. RALs were estimated as 0.78 ± 0.04 adducts per 10^{-7} total nucleotides. Based on the chromatographic behavior of one of the identified adducts, the authors suggested that it was derived from TCpBQ.

The study noted that PCP-induced DNA adducts have been found at much higher occurrences (adduct levels of 8×10^{-7}, 3.2×10^{-7}, and 1.7×10^{-6} for PCP, TCHQ with HRP and H_2O_2, and TCBQ, respectively) in mouse liver (Bodell and Pathak, 1998), possibly as a consequence of higher amounts of PCP quinone metabolites found in mouse liver as compared with rat liver (Lin et al., 1997). PCP formed direct DNA adducts in vitro with HRP and H_2O_2, but formed DNA adducts in vivo only after dehalogenation and quinone formation (Lin et al., 2002).

4.5.3. Protein Adduct Formation

NTP (1999) reported protein adducts of chlorinated quinones and semiquinones in tissue samples from F344 rats after 7 months of dosing with 1,000 ppm (60 mg/kg-day) dietary aPCP (99% purity). The level of hemoglobin adducts was elevated in male and female rats.

Lin et al. (1999) investigated the production of chlorinated quinone and semiquinone adducts in the livers of Sprague-Dawley rats and B6C3F1 mice following a single oral dose of 0-40 mg/kg PCP and in male Fischer 344 rats following chronic ingestion of 60 mg PCP/kg for 6 months. At low PCP doses (<4–10 mg/kg), TCoSQ-protein adduct formation in liver cytosol and nuclei was higher in rats than in mice. At high PCP doses (>60–230 mg/kg), however,

TCpBQ adducts were higher in mice than in rats. Moreover, there was a fourfold difference in the nuclear total of quinone metabolites in the mouse compared with that in the rat (Lin et al., 1997). Lin et al. (1999) speculated that such differences in the metabolism of PCP to semiquinones and quinones might be responsible for the production of liver tumors in mice but not rats.

Waidyanatha et al. (1996) examined adducts to blood proteins, albumin and hemoglobin, in three male Sprague-Dawley rats/dose treated with a single dose (gastric intubation) of 5, 10, 20, or 40 mg/kg aPCP (99% purity). Rats were sacrificed 24 hours following administration of PCP. Protein adducts involving reactive metabolites of PCP, TCpBQ (specifically mono-, di-, and tri-substituted forms of chlorinated benzoquinones), TCpSQ, and TCoSQ were identified for both albumin and hemoglobin following administration of PCP. TCoBQ adducts were not identified in the blood of the rats in this study. The authors performed a linear regression for each of the hemoglobin and albumin adducts in vivo as pM adducts per mg PCP/kg rat body weight and reported the resulting slopes.

The benzoquinone adducts were detected at greater concentrations in albumin compared with hemoglobin, while the semiquinones were present in greater amounts in hemoglobin. The greatest concentration of adducts was observed with the tri-substituted benzoquinone, Cl_3BQ-Y (whereY represents the protein). For the adducts Cl_3BQ-Y, 2,3-Cl_2BQ-Y2, 2,5-and 2,6-Cl_2BQ-Y2, ClBQ-Y3, TCoSQ-Y, and TCpSQ-Y, the slopes were reported as 79 ± 8.84, 11.4 ± 1.3, 8.28 ± 1.18, ND, 47.9 ± 3.44, and 20.2 ± 4.04 for formation in hemoglobin, respectively, and 200 ± 13.3, 14.2 ± 1.65, 8.75 ± 0.33, 1.06 ± 0.065, 13.9 ± 1.47, and 13.7 ± 0.98 for formation in albumin, respectively. Based on the observed proportional relationship between the adduct levels and TCpBQ, the authors concluded that the adducts were produced in a dose-dependent manner following administration of PCP. These results provided further evidence that PCP administered to rodents results in the formation of adducts via the oxidative dechlorination of PCP to reactive quinones and semiquinones.

In a second experiment, Waidyanatha et al. (1996) administered a single dose via gastric intubation of 20 mg/kg aPCP to three male Sprague-Dawley rats/group to investigate the stability of PCP-induced protein adducts. The eight groups of rats were characterized by the duration of time between treatment and sacrifice of 0, 2, 4, 8, 24, 48, 168, or 336 hours. Following 8 and 24 hours, the adduct levels achieved a maximum concentration and declined at times exceeding 24 hours. Two adducts were presented to serve as a representative measurement for the remaining identified adducts. The di- and tri-substituted benzoquinones, 2,3-Cl_2BQ-Y_2 and Cl_3BQ-Y, reached maximum levels of 8 and 60 pmol/g for hemoglobin and 150 and 800 pmol/g for albumin, respectively (values were estimated and extracted from a graph). Elimination half-lives for these adducts were calculated as 155 and 41 hours for the hemoglobin and albumin adducts, respectively. Both of these durations are shorter than the normal rate of turnover for both

erythrocytes and serum albumin. The authors suggested that the adducts identified in vivo were somewhat unstable and attributed this to continuing sulfhydryl group reactions.

The available DNA and protein adduct studies provide further evidence that PCP, or more specifically the quinone (hydro- or benzo-) and semiquinone metabolites of PCP, can interact with DNA in rodents. Furthermore, the liver, considered to be the target organ of both noncancer toxicity and carcinogenicity, is susceptible to DNA alteration via PCP exposure and the subsequent formation of DNA and/or protein adducts.

4.5.4. Oxidative DNA Damage and 8-Hydroxy-2'-Deoxyguanosine Formation

4.5.4.1. *In Vitro Studies*

Reactive oxygen species (ROS) generated by metabolic processes may have a role in PCP-induced oxidative DNA damage. Research initiatives have focused on the question of whether ROS and/or biological reactive intermediates (BRIs) were the ultimate causative agents in DNA damage and cancer.

Carstens et al. (1990) reported an increase in SSBs in DNA of cultured human fibroblasts following administration of 50 μM TCHQ. They observed highly effective suppression in TCHQ-induced SSBs in presence of the hydroxyl radical scavengers, dimethyl sulfoxide (DMSO), ethanol, or mannitol; the metal chelator, deferoxamine; and the enzyme catalase. The metal chelator diethylenetriamine pentaacetic acid (DETAPAC) and enzyme superoxide dismutase (SOD) had little effect on the TCHQ-induced SSBs. DMSO was similarly effective in preventing DNA breakage induced by 10 or 30 μM TCHQ in cultured human fibroblasts. The researchers used electron spin resonance to show that the tetrachlorosemiquinone radical, an autoxidation product of TCHQ, was present in the reaction mixtures at up to 60% of the original TCHQ concentrations. Formation of this radical entails the production of superoxide radicals that produce hydroxyl radicals. The low efficiency of SOD and DETAPAC, which block the iron-catalyzed Haber-Weiss reaction of the superoxide radical, was seen as an indication that the superoxide radical plays a minor role in TCHQ-induced DNA damage. However, the suppressive effect that deferoxamine, which blocks the semiquinone radical-driven Fenton reaction, had on the SSBs indicated that the semiquinone radical was the major DNA-damaging agent. The high efficiency of the hydroxyl radical scavengers, however, suggested also an important function for the hydroxyl radical. Thus, both ROS and BRI were involved in TCHQ-induced DNA damage.

Lin et al. (2001a) found a dose-dependent increase in the number of apurinic (AP) sites following incubation of calf thymus DNA with 1, 2.5, or 5 mM TCpBQ. The increase over control was roughly threefold at 5 mM TCpBQ. In another experiment, 1 or 10 μM TCpBQ was incubated with calf thymus DNA in the presence of 100 μM NADPH and 100 μM Cu(II) to determine if ROS formed from the redox cycling of TCpBQ induced by the reducing agent, NADPH, and copper resulted in the AP sites previously observed with TCpBQ. At the μM

concentrations, much lower than previous concentrations (e.g., 1, 2.5, or 5 mM), TCpBQ with NADPH and Cu(II) induced statistically significant increases in the AP sites when compared with control. Approximately 5- and 10-fold increases in AP sites were observed with 1 and 5 μM TCpBQ, respectively, in the presence of NADPH and Cu(II). The authors suggested that this effect could be attributed to redox cycling of TCpBQ.

Similar experiments with 300 μM TCpHQ showed no increase in AP sites, although the addition of 100 μM Cu(II) resulted in a sixfold increase (10.8 ± 0.5 AP sites/105 nucleotides) over control (1.6 ± 0.2 AP sites/105 nucleotides). The increase in AP sites observed with TCpHQ and Cu(II) was dose-dependent for concentrations of TCpHQ from 0.5 to 300 μM. Additionally, the number of AP sites was reduced with the addition of 5U catalase, suggesting that hydrogen peroxide was involved in the formation of the AP sites (Lin et al., 2001).

Jansson and Jansson (1992) showed a significant induction of micronuclei in V79 Chinese hamster cells treated with 10, 15, and 20 μM TCHQ (>99% purity). Combined administrations of TCHQ with DMSO (a hydroxyl radical scavenger) and ethyl methanesulfonate (EMS; an alkylating agent) and DMSO were performed to determine if hydroxyl radicals were involved in the TCHQ-induced chromosomal damage. A 5% solution of DMSO combined with 15 μM TCHQ partially inhibited the micronucleus formation observed with TCHQ alone. Because DMSO did not similarly inhibit the formation of micronuclei following EMS treatment, the authors concluded that these results provide support for a role of hydroxyl radicals in the chromosomal damage associated with TCHQ.

Lin et al. (2001) assayed calf thymus DNA treated with TCpBQ to determine if the benzoquinone induced changes in the levels of oxidative DNA damage indicator 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and whether these changes were related to TCpBQ-induced AP sites. While the control measurement of 8-OH-dG was high (the authors treated this as "an artifact of commercial isolation"), the levels of 8-OH-dG increased in a statistically significant, dose-dependent fashion. Approximately 2-, 2.5-, and 3-fold increases in 8-OH-dG per 10^5 dG were observed with 1, 2.5, and 5 mM of TCpBQ. This change in 8-OH-dG occurred parallel to formation of AP sites, leading the authors to suggest that the AP sites formed as a result of oxidative stress-induced DNA damage. Additionally, parallel increases in SSBs were dose-dependent, with amplified DNA fragmentation at 1 and 10 μM TCpBQ in the presence of Cu(II) and NADPH, but not with 5 mM TCpBQ alone.

TCpHQ, at concentrations ranging from 0.5 μM to 1 mM, incubated with calf thymus DNA failed to induce 8-OH-dG compared with controls. However, the addition of 100 μM Cu(II) to TCpHQ resulted in a statistically significant, dose-dependent increase in 8-OH-dG. TCpHQ (with 100 μM Cu(II)) at a concentration of 300 μM produced a threefold increase in 8-OH-dG per 105 dG compared with controls. The authors suggested that the metal facilitated TCpHQ autooxidation, generating ROS and subsequently oxidative DNA damage. Additionally,

dose-dependent increases in DNA SSBs were observed parallel to increased 8-OH-dG levels (Lin et al., 2001).

Naito et al. (1994) investigated the mechanism of PCP metabolite-induced DNA damage in vitro. They incubated TCHQ with calf thymus DNA in the presence or absence of cations (Cu^{2+}, Mn^{2+}, or Fe^{3+}) that are known to be involved in redox cycling, and found that Cu^{2+} facilitated 8-OH-dG formation in the presence of TCHQ. This effect was not suppressed by typical hydroxyl scavengers but was abolished by bathocuproine (a Cu^{+} chelator) or catalase, from which the authors concluded that Cu^{+} and H_2O_2 were involved in the production of reactive species causing DNA damage. The authors concluded that it was not the semiquinone, but rather redox cycling with superoxide and H_2O_2 formation and the subsequent metal-catalyzed decomposition into hydroxyl radicals that played the crucial role in oxidative DNA damage.

Dahlhaus et al. (1995) treated Chinese hamster V79 lung fibroblasts with 0, 6.25, 12.5, 25, or 50 µM TCpHQ for 1 hour and measured 8-OH-dG formation immediately or up to 2 hours after treatment. After normalizing for variable background levels of 8-OH-dG in control V79 cells, they found that 25 and 50 µM (but not 6.25 and 12.5 µM) caused approximately two-fold increases in 8-OH-dG. The 25 µM concentration was associated with low cytotoxicity, while the 50 µM concentration exhibited appreciable cytotoxicity. The increase in 8-OH-dG correlated with the cytotoxicity at 25 µM, although 50 µM presented similar levels of 8-OH-dG as observed with the lower dose. The increase in 8-OH-dG formation was optimal after 1 hour of TCpHQ exposure, but was much reduced after 2 hours of exposure. The authors suggested that this was a sign of activation of a repair system in the V79 cells.

Dahlhaus et al. (1996) investigated PCP, TCpHQ, TCpBQ, TCoHQ, and TCoBQ for the ability to produce oxidative DNA damage in Chinese hamster V79 cells. Changes in 8-OH-dG in the DNA of the V79 cells were examined after exposure for 1 hour to 25 µM PCP or one of its metabolites. TCpHQ, TCpBQ, and TCoBQ produced 8-OH-dG at levels approximately 2- to 2.5-fold greater than those observed with either PCP or the control. TCoHQ and PCP did not show an increase in 8-OH-dG. The authors discussed their findings in terms of redox cycling leading to ROS (i.e., direct attack of hydroxyl radicals, excision repair of hydroxylated DNA bases, or cytotoxic effects) as the possible causes of this DNA damage.

As a means of further investigating the mechanism of redox cycling by PCP metabolites, electron spin resonance (ESR) spin trapping was used by Zhu and Shan (2009) to identify the metabolite involved in producing hydroxyl radicals. Previous work (Zhu et al., 2000) using the salicylate hydroxylation method had shown that in the presence of hydrogen peroxide, both TCpHQ and TCpBQ were able to produce hydroxyl radicals in a metal-independent reaction, implicating an alternate pathway to the Fenton reaction in producing these radicals. Based on the reaction products, the authors determined that a novel mechanism involving a nucleophilic reaction between TCpBQ and hydrogen peroxide leads to the formation of an unstable hydroperoxyl-1,4-benzoquinone intermediate, which decomposes homolytically to produce

hydroxyl radicals and trichloro-hydroxy-1,4-benzoquinone radicals. However, it is not been determined if this reaction has relevance in vivo.

4.5.4.2. *In Vivo Studies*

Lin et al. (2002) administered PCP (purity not reported, although likely aPCP as authors compared results to NTP [1999] which used aPCP, and earlier studies by Lin et al. [1999, 1997] used aPCP) to groups of three or four male F344 rats at concentrations of 30, 60, or 120 mg/kg-day for 1 day and concentrations of 30 or 60 mg/kg-day for 5 days. Additionally, Lin et al. (2002) obtained tissues from the livers of 10 F344 rats fed 60 mg/kg-day aPCP for 27 weeks in a 2-year bioassay conducted by NTP (1999). The induction of the 8-OH-dG lesion in rat liver DNA was evaluated for the rats exposed to aPCP. There was no induction in 8-OH-dG at the 30, 60, or 120 mg/kg-day dose groups treated with PCP for 1 or 5 days when compared with controls. However, there was a statistically significant increase ($1.8 \pm 0.65 \times 10^{-6}$) in the level of 8-OH-dG per 10^6 dG that was twofold greater in rats fed 60 mg/kg-day aPCP for 27 weeks compared to controls ($0.91 \pm 0.42 \times 10^{-6}$). Lin et al. (2002) noted that the liver adducts observed in another assay were present at levels well below (10-fold lower) the 8-OH-dG concentration. However, it was observed that two distinct types of DNA adducts formed in parallel to the 8-OH-dG lesions in the liver of rats chronically administered PCP. DNA adducts were also detected in rat kidney, but at levels 10-fold lower than the adducts and 8-OH-dG lesions in the liver.

Sai-Kato et al. (1995) studied the influence of PCP on the formation of 8-OH-dG in the liver of $B6C3F_1$ mice administered PCP by gavage at 30, 60, or 80 mg/kg as a single dose or five consecutive doses to groups of 5 male mice. A clear dose-response relationship was also observed with both treatments (no specific trend analysis was described). The 8-OH-dG formation after a single dose (1.4- and 1.7-fold at 60 and 80 mg/kg, respectively) and repeated exposures (1.5-, 1.9- and 1.9-fold at 30, 60, or 80 mg/kg-day, respectively) was statistically significantly increased compared with controls. Formation of 8-OH-dG was specific for the target organ, liver; no significant increase in 8-OH-dG levels was observed in kidney or spleen. Based on evidence of the presence of a repair enzyme for 8-OH-dG in mammalian cells (Yamamoto et al., 1992), the finding that elevation of 8-OH-dG levels was not observed at 24 hours after a single i.p. injection of an 80 mg/kg dose of PCP suggests that repair of this oxidative DNA damage had occurred by that time point. However, single administration via gavage and repeat administration of PCP caused elevated levels of 8-OH-dG at low doses (30 or 60 mg/kg-day). The authors concluded that long-term exposure of PCP may induce gradual accumulation of oxidative DNA damage in the liver by overwhelming the repair potential and that this cumulative oxidative DNA damage could cause critical mutations leading to carcinogenesis (Sai-Kato et al., 1995).

Umemura et al. (1996) demonstrated that feeding aPCP (98.6% purity) to male $B6C3F_1$ mice for 2 or 4 weeks at concentrations of 41, 86, and 200 mg/kg-day resulted in dose-dependent, statistically significant two- to threefold increases of 8-OH-dG formation in the liver. In addition to the dose- and time-dependent elevation of 8-OH-dG, significantly elevated bromodeoxyuridine (BrdU) labeling index and hepatic DNA content (indicative of hyperproliferation) led the authors to suggest that oxidative DNA damage in combination with hyperproliferation might cause PCP-related cancer.

Umemura et al. (1999) fed mice 600 or 1,200 ppm PCP (98.6% purity; doses are estimated as 108 and 216 mg/kg-day, respectively) for 8 weeks and noted that the oxidative lesion 8-OH-dG in liver DNA was statistically increased to 2.5- and 3.8-fold at 108 and 216 mg/kg-day, respectively, compared with the control levels. La et al. (1998a) reported that F344 rats fed PCP for 27 weeks showed a twofold increase in the 8-OH-dG DNA lesion in liver. Another lesion was noted and compared with in vitro PCP metabolite adducts. This lesion co-migrated with the TCpBQ adduct but at an absolute level threefold lower than that of the oxidative lesion.

Dahlhaus et al. (1994) showed that the PCP metabolite TCpHQ elicited an approximately 50% increase in 8-OH-dG formation in hepatic DNA of $B6C3F_1$ mice fed 300 mg/kg TCpHQ for 2 or 4 weeks. Single i.p. injections of 20 or 50 mg/kg TCpHQ had no such effect.

4.5.5. Uncoupling of Oxidative Phosphorylation

The ability of PCP to uncouple mitochondrial oxidative phosphorylation was first described by Weinbach (1954). This study measured the net uptake of phosphate and oxygen in rat liver mitochondria during the oxidation of α-ketoglutarate to succinate to indicate the extent of oxidative phosphorylation uncoupling. PCP induced the uncoupling of oxidative phosphorylation in a dose-dependent manner. At the lowest concentration tested, 10^{-6} M, PCP showed signs of suppressing phosphate uptake, but this was accompanied with a stimulation of oxidation. However, at concentrations of 10^{-5} and 10^{-4} M, PCP suppressed phosphate uptake while having little effect on oxidation, indicative of uncoupling as respiration was being stimulated without concomitant phosphorylation. At concentrations of 10^{-3} M and higher, PCP completely inhibited both phosphorylation and oxidation. PCP also accelerated the breakdown of mitochondrial ATP, which the author theorized was a consequence of altered membrane permeability, as PCP had a suppressive effect on ATPase (Weinbach, 1954).

Arrhenius et al. (1977a) observed that PCP, not a metabolite, exerted a strong inhibition of electron transport between a flavin coenzyme and CYP450. In the second part of that study, Arrhenius et al. (1977b) looked at the effects of PCP on cellular detoxification mechanisms. Their main focus was to examine whether PCP acts only as an inhibitor of oxidative phosphorylation in mitochondria or if it exerts an additional effect on the microsomal electron transport. The experiments were conducted in vitro with the subcellular fraction from liver of

male Wistar rats, using oxygen consumption as the measure of respiration. PCP was about twice as potent in mitochondria as the commonly used uncoupler, dinitrophenol. The authors concluded that the parent compound, not a metabolite, was the active toxicant and that it inhibited the electron transport from flavin to CYP450. The authors discussed their findings in terms of a possible effect of lipophilic chlorophenols on membrane function.

Varnbo et al. (1985) used a murine neuroblastoma-derived cell line to investigate the influence of a variety of toxicants on respiratory activity as measured by oxygen consumption. aPCP was used at concentrations between 100 µM and 1 mM and caused a brief spike in oxygen consumption followed by a dose-dependent decrease that reached approximately 70% inhibition within 30 minutes at 1 mM aPCP.

A series of experiments was conducted with female Wistar rats that were fed 0.2% HCB in the diet for up to 60 days (Trenti et al., 1986a, b; Masini et al., 1985, 1984a, b). PCP is chemically similar to HCB, which is a benzene ring with a chlorine bound to each of the six carbons. In the PCP molecule, one chlorine atom present in HCB is replaced with a hydroxyl (OH) group, rendering the molecule somewhat electrophilic. One of the pathways for HCB metabolism produces PCP. Animals were sacrificed at 20, 40, and 60 days of feeding, and mitochondria were prepared from their livers. Masini et al. (1984a) observed that the porphyrin content of liver mitochondria increased with time, but porphyrins were not detectable in urine or feces. Using oligomycin, the authors found that the change in ratio of state 3 to state 4 respiration (i.e., respiratory control index) was due to uncoupling of oxidative phosphorylation. The effect was reversible by addition of BSA, a scavenger for uncoupling agents. The authors speculated that phenolic metabolites of HCB, specifically PCP, caused the uncoupling of oxidative phosphorylation.

Masini et al. (1984b) recorded the transmembrane potentials of mitochondria from HCB-treated animals and control mitochondria with added micromolar concentrations of PCP and found that they were highly similar. Subsequently, the same investigators (Masini et al., 1985) reported a time-dependent increase, up to 600-fold, of porphyrins in the urine, liver, and mitochondria of female Wistar rats. PCP levels in livers and liver mitochondria of HCB-treated animals rose with time in parallel with HCB levels, amounting to about 10% of the HCB load per gram of liver tissue, and per mg protein (liver mitochondria). To strengthen their hypothesis that the HCB metabolite PCP might be responsible for the observed effects, these researchers added PCP to a mitochondrial suspension at 0.25–2.5 µM, which caused a dose-dependent inhibition of oxidative phosphorylation that was reversible by the addition of BSA.

Trenti et al. (1986a) found that oxygen usage per mg mitochondrial protein was almost doubled by treatment with either 0.2% HCB or 1 µM PCP. The effect was fully reversible by the addition of 0.1% BSA to the medium. The authors concluded that the increased oxygen usage observed after HCB feeding was entirely caused by the HCB metabolite, PCP. In a parallel experiment, Trenti et al. (1986b) fed female Wistar rats with 0.2% HCB in the diet for up to

60 days and prepared mitochondria from their livers after 20, 40, and 60 days of feeding. There was a constant decline in the respiratory control index (ratio of state 3 to state 4 respiratory rate), the ADP:oxygen ratio, and the transmembrane potential with time. The investigators also observed that PCP concentrations in liver and mitochondria increased with time, paralleled by an increase in porphyrins. However, they concluded that porphyrin formation was unrelated to uncoupling of oxidative phosphorylation.

4.5.6. Cytotoxicity

Freire et al. (2005) evaluated the potential cytotoxic effects of PCP on Vero monkey cells (from the kidney of the African green monkey) by incubating cultures with PCP concentrations of 1, 5, 10, 50, or 100 μM (0.26–26.63 μg/mL) for 24, 48, or 72 hours. There was a statistically significant increase in cytotoxicity at the 5 μM concentration of PCP with cell viabilities of 72, 70, and 45% of the control for the 24-, 48-, and 72-hour incubation periods, respectively. The cytotoxicity increased in a dose- and time-dependent manner. The viabilities of the Vero cells measured at the higher concentrations of PCP were <40% of the control for all three incubation periods.

Additionally, Freire et al. (2005) looked at effects on lysosomes and mitochondria in cells incubated with 10, 40, or 80 μM PCP for 3 or 24 hours. Damaged lysosomes or a reduced number of intact lysosomes increased in a dose- and time-dependent manner. Large vacuoles, potentially indicative of lysosomal fusion or swelling, were observed at all doses after 24 hours. A disturbance in the transmembrane potential of the mitochondria in the Vero cells was observed after 3 hours of incubation with the 40 and 80 μM dose groups of PCP. After 24 hours, the cells exhibited severely compromised mitochondria (with 80 μM) and statistically significant morphological changes (chromatin condensation and nuclear fragmentation) that were indicative of apoptosis (with all doses).

Dorsey et al. (2004) incubated alpha mouse liver 12 (AML 12) hepatocytes with PCP at concentrations of 1.95, 3.95, 7.8, 15.6, or 31.2 μg/mL (98% purity) for 48 hours to examine the cytotoxic effects of PCP. The viability of the cells treated with the lower doses (≤7.8 μg/mL) was greater than that measured with the control; however, at the two highest doses, 15.6 and 31.2 μg/mL, cell viability was statistically significantly reduced by >50% compared with controls. Additionally, the authors examined morphology of the AML 12 hepatocytes following incubation with PCP. Morphologic effects were observed as changes in cell shape and in the monolayer after 48 hours of incubation with 15.6 μg/mL PCP.

In the same study, Dorsey et al. (2004) looked at the mitogenic effects of 0.975, 1.95, 3.95, or 7.8 μg/mL PCP on AML 12 hepatocytes after 12 and 24 hours of incubation. Stimulatory patterns of cell proliferation in treated heptocytes were compared with untreated cells. Cell proliferation was statistically significantly increased one- to threefold at all doses and

both durations of incubation with PCP. The authors noted that PCP was mitogenic at low doses in the AML 12 mouse hepatocytes.

This group also observed, in previous studies, dose-dependent cytotoxic effects in HepG2 cells (LD_{50} = 23.0 ± 5.6 μg/mL) with decreased viabilities that were 95, 90, 40, 30, and 10% of the control following incubation with 6.25, 12.5, 25, 50, or 100 μg/mL PCP, respectively, for 48 hours (Dorsey and Tchounwou, 2003). The decreased cell viability was statistically significant at all doses except the lowest dose of 6.25 μg/mL. PCP exerted mitogenic effects on HepG2 cells with one- to five-fold increases in cell proliferation at doses ranging from 0.20 to 3.25 μg/mL (Dorsey and Tchounwou, 2003). Suzuki et al. (2001) observed cytotoxicity, measured by release of LDH from Wistar rat hepatocytes. Cytotoxicity was significantly increased (20–35% release of LDH) following incubation with 1 mM PCP for 1 hour compared with controls.

4.5.7. Lipid Peroxidation

Suzuki et al. (2001) isolated Wistar rat hepatocytes and incubated them for 1 hour with 1 mM PCP (purity not reported) to examine the lipid peroxidative and cytotoxic effects. PCP induced a slight but statistically significant increase in cellular phospholipoperoxides. Additionally, glutathione was nearly depleted with administration of PCP. The authors suggested that this depletion may have induced the lipid peroxidation.

4.5.8. Inhibition of Gap Junction Intercellular Communication

Sai et al. (1998) investigated the possible role that inhibition of gap junction intercellular communication (GJIC), a nongenotoxic mechanism, may play in contributing to tumor promotion. They used WB-F344 rat epithelial cell lines with concentrations ranging from 25 to 200 μM PCP (≤24 hours) and TCHQ (1 hour). Incubations with PCP at concentrations >40 and >75 μM for TCHQ were found to induce cytotoxicity. Subsequent GJIC experiments were conducted under conditions that did not elicit cytotoxicity. A time course of GJIC inhibition by PCP revealed a 40% inhibition by 4 hours, a return to normal levels by 6–8 hours, and a second phase of inhibition up to 50%, lasting from 16–24 hours. The effect displayed dose-dependence from 10 to 40 μM PCP. When cells were incubated with 20 or 40 μM PCP for 4 or 24 hours and then reincubated in the absence of PCP, normal GJIC was restored within 4–6 hours. Four hours of exposure to 40 μM PCP significantly reduced the levels of connexin (CX43), a GJIC-specific protein, in WBCs but did not affect its localization on the cell surface. Removal of PCP restored CX43 levels within 6 hours. Phosphorylation of CX43 was not affected by 40 μM PCP, while strong phosphorylation was achieved by the potent tumor promoter, tetradecanoylphorbol acetate (TPA) (concentration not stated). The authors concluded that the PCP-induced GJIC inhibition was not based on changes in CX43 phosphorylation, but more likely represented a

posttranslational event. TCHQ did not affect GJIC in WBCs, but it is possible that the time of exposure (1 hour) was too short to elicit measurable changes.

In a subsequent study, Sai et al. (2000) administered green tea (in place of drinking water) for 3 weeks to male B6C3F_1 mice. For the latter 2 weeks of treatment, the animals were exposed to 300 or 600 ppm PCP (doses estimated as 54 and 108 mg/kg-day, respectively) via feed [these doses were chosen because they had demonstrated tumor-promoting activity in an initiation-promotion assay (Umemura et al., 1999)]. PCP alone inhibited GJIC up to 60% in a dose-dependent manner; a similar, albeit reduced inhibition (maximally 10%) was observed in the animals co-treated with green tea. Expression of CX32, another GJIC-specific marker, on the cytoplasmic membrane was attenuated by PCP treatment. This effect was prevented by green tea treatment.

Exposure to 54 and 108 mg/kg-day PCP in feed for 2 weeks increased cell proliferation (as evidenced by the BrdU labeling index) 6- and 15-fold, respectively, compared with controls. Co-treatment with green tea lessened this proliferative effect by 60–70%. Because green tea contains highly effective antioxidants, the authors suggested that PCP caused GJIC inhibition by means of oxidative stress. They did not elaborate as to whether the formation of oxygen radicals and oxidative stress required metabolism of PCP (Sai et al., 2000).

Sai et al. (2001) conducted another study of the effects of aPCP on GJIC in which they evaluated possible mechanistic links to apoptosis, using a WB-F344-derived rat epithelial cell line. An aPCP concentration of 2 µM was chosen for the tests based on the observation that 1 µM was minimally effective, while 3 µM marked the beginning of cytotoxicity. Apoptosis was induced by serum deprivation of the cultured cells, which takes 3–6 hours to first become evident in the form of cell detachment from the dish and is at a maximum by 12 hours after serum removal. Three different methods were used: apoptosis staining using Hoechst 33342; the terminal deoxynucleotidyltransferase mediated deoxyuridine 5'-triphosphate-biotin nick-end labeling (TUNEL) test; and DNA ladder formation. By all three measures, aPCP inhibited serum deprivation-induced apoptosis at 2 µM in a time-dependent manner. While serum deprivation alone did not affect GJIC until 12 hours after removal, aPCP caused a significant inhibition of GJIC within 1 hour. Additionally, aPCP caused up to a 60% drop in the protein level of p53, an apoptosis-inducing protein, in the serum-deprived cells over a period of 12 hours. Subsequent decreases in mRNA levels of p53 were observed, as well as a similar decrease in the level of GJIC-specific CX43. The authors considered these findings evidence that aPCP inhibits GJIC formation that would be required for propagation of the “death signal,” thus preventing apoptosis and the elimination of transformed cells. The aPCP-induced effects on p53 and CX43 may explain the decrease in apoptosis and GJIC. It was suggested that the suppression of apoptosis and GJIC could lead to tumor promotion.

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

The liver is the primary target for noncancer effects of oral exposure to PCP. Numerous short- and long-term oral studies show that PCP is toxic to the liver of rats, mice, and dogs (see Table 4-23). Liver toxicity is generally manifested by increased absolute and relative weights and a wide spectrum of microscopic lesions. Liver toxicity in long-term studies in rats was primarily characterized by pigment accumulation (Schwetz et al., 1978), chronic inflammation at high doses, and cystic degeneration at lower doses in males (NTP, 1999); female rats were not as sensitive as males in the NTP study. Liver toxicity in mice exposed orally to PCP was manifested primarily by necrosis, cytomegaly, chronic active inflammation, and bile duct lesions (NTP, 1989). Liver toxicity was more severe in mice than rats at similar doses, which could be partially attributable to differences in biotransformation of PCP. Additionally, rats in one of the chronic studies (NTP, 1999) were treated with aPCP, whereas mice in the chronic NTP (1989) study received either tPCP or EC-7 grades of PCP, which are higher in chlorinated dibenzo-p-dioxins and dibenzofuran contaminants and may contribute to the severity of the response in mice compared with rats. NTP (1989) studies showed very little difference between the toxicity of tPCP and EC-7 in mice, except for bile duct hyperplasia, which may be associated with the impurities in tPCP. Liver lesions in the dog (Mecler, 1996) were similar to those observed in the mouse (NTP, 1989), but the doses inducing the lesions in the dog were lower than those that induced these lesions in the mouse (1.5 mg/kg-day compared with 17–18 mg/kg-day for the mouse). Studies in domestic animals showed that pigs, but not cattle, exhibited liver lesions similar to those observed in mice. The pig exhibited liver toxicity at a lower dose (10 versus 17–18 mg/kg-day for the mouse) and for a shorter duration (30 days versus 2 years) than the mouse. Other noncancer targets identified in long-term studies include the kidney (pigment deposition in the proximal convoluted tubules) of rats (Schwetz et al., 1978) and the spleen (decrease in organ weight) of mice (NTP, 1989), rats (Bernard et al., 2002), and calves (Hughes et al., 1985).

Table 4-23. Subchronic, chronic, developmental, and reproductive oral toxicity studies for PCP

Species/strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Effect(s) at the LOAEL	Reference
Subchronic						
Mice, Swiss-Webster (6 females/dose)	0, 10, 51, or 102 (feed) 8 weeks	tPCP	10	51	Dose-related increases in hepatocellular multifocal necrosis, hepatocellular and nuclear swelling, hepatocellular vacuolation, and eosinophilic inclusion bodies in nuclear vacuoles.	Kerkvliet et al., 1982a[c]
Mice, B6 (15–16 female mice/dose)	0, 10, 20, or 49 (feed) 8 weeks	aPCP	10	20		
Mice, B6 (20 males/dose)	0, 10, or 98 (feed) 12 weeks	tPCP	ND	10	Dose-related increases in hepatocellular swelling, nuclear swelling and vacuolation with eosinophilic inclusion bodies.	Kerkvliet et al., 1982b[c]
		aPCP				
Rat, Wistar weanlings (10/sex/dose)	0, 2, 5, 18 (M) (feed) 12 weeks	tPCP	2	5	Centrilobular vacuolation[b], increased aniline hydroxylase activity in liver microsomes.	Knudsen et al., 1974
	0, 3, 5, 21 (F) (feed) 12 weeks		3	5		
Rat, Sprague-Dawley (number not reported)	0, 3, 10, or 30 (feed) 90 days	Commercial	ND	3	Dose-related elevated serum ALP and increases in liver and kidney weight.	Johnson et al., 1973[c]
		Improved	3	10	Increased liver weight	
		Pure	3	10		
Rat (10 males/dose)	0 or 87 (feed) 90 days	tPCP	ND	87	Enlarged liver, single hepatocellular necrosis, hepatocellular vacuolation, cytoplasmic inclusion, slight interstitial fibrosis, brown pigment in macrophages and Kupffer cells, atypical mitochondria.	Kimbrough and Linder, 1975[c]
		aPCP			Enlarged liver, hepatocellular vacuolation, cytoplasmic inclusion, atypical mitochondria.	

Table 4-23. Subchronic, chronic, developmental, and reproductive oral toxicity studies for PCP

Species/strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Effect(s) at the LOAEL	Reference
Rat, male Wistar (number not reported)	0, 80, 266, or 800 mg/L (drinking water) 60–120 days	Not reported	80	266	Dose-related increases in hepatocellular degeneration and necrosis, increased granular endoplasmic reticulum, congested portal veins, enlarged and congested sinusoids, and bile duct hyperplasia. Nephritis in kidney including glomerular congestion and hyalinization.	Villena et al., 1992[c]
Mice, $B6C3F_1$ (25 males/dose; 10 females/dose)	0, 38, or 301 (M) (feed) 26–27 weeks	tPCP	ND (M)	38 (M)	Dose-related increases in incidence and severity of liver lesions including hepatocellular degeneration and necrosis, karyomegaly, and cytomegaly.	NTP, 1989[c]
	0, 52, or 163 (F) (feed) 26–27 weeks		ND (F)	52 (F)		
	0, 36, 124, or 282 (M) (feed) 26–27 weeks	EC-7	ND (M)	36 (M)		
	0, 54, 165, or 374 (F) (feed) 26–27 weeks		ND (F)	54 (F)		
	0, 40, 109, or 390 (M) (feed) 26–27 weeks	DP-2	ND (M)	40 (M)		
	0, 49, 161, or 323 (F) (feed) 26–27 weeks		ND (F)	49 (F)		
	0, 102, 197, or 310 (M) (feed) 26–27 weeks	aPCP	ND (M)	102 (M)		
	0, 51, 140, or 458 (F) (feed) 26–27 weeks		ND (F)	51 (F)		

Table 4-23. Subchronic, chronic, developmental, and reproductive oral toxicity studies for PCP

Species/strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Effect(s) at the LOAEL	Reference
Chronic						
Rat, Sherman (10/sex/dose)	0, 2, 9, or 44 (M) 0, 2, 10, or 48 (F) (feed) 8 months	tPCP	ND	2	Dose-related increases in centrolobular hepatocyte hypertrophy and vacuolation; at higher doses, pleomorphism, bile duct proliferation, adenofibrosis, cytoplasmic hyaline inclusions, abundant brown pigment in macrophages and Kupffer cells, and statistically significantly increased liver weight.	Kimbrough and Linder, 1978[c]
		aPCP	9 (M) 10 (F)	44 (M) 48 (F)	Statistically significant decrease in body weight, slight hepatocyte hypertrophy, eosinophilic cytoplasmic inclusions, and brown pigment in liver.	
Dog, beagle (4/sex/dose)	0, 1.5, 3.5, or 6.5 (gelatin capsule) 1 year	tPCP	ND	1.5	Dose-related increases in incidence and severity of hepatocellular pigmentation, cytoplasmic vacuolation, chronic inflammation; significantly increased serum ALT and AST; significantly increased relative liver weight; and increased absolute liver wt (significant in females).	Mecler, 1996[c]
Rat, F344 (50/sex/dose)	0, 10, 20, or 30 (feed) 2 years	aPCP	10 (M)	20 (M)	Increased cystic degeneration[b] and decreased body weight.	NTP, 1999[c]
			20 (F)	30 (F)	Decreased body weight.	
Rat, Sprague-Dawley (25/sex/dose)	0, 1, 3, 10, or 30 (feed) 2 years	EC-7	10 (M)	30 (M)	Dose-related increases in pigmentation in liver.	Schwetz et al., 1978
			3 (F)	10 (F)	Dose-related increases in pigmentation in liver and kidney.	
Mouse, B6C3F$_1$ (50/sex/dose)	tPCP: 0, 18, or 35 (M); 0, 17, or 35 (F) EC-7: 0, 18, 37, or 118 (M); 0, 17, 34, or 114 (F) (feed) 2 years	tPCP/EC-7	ND	18 (M)	[b]Increased clear cell focus, acute diffuse necrosis, diffuse cytomegaly, diffuse chronic active inflammation, multifocal accumulation of brown pigmentation (LF and cellular debris) in Kupffer cells in the liver, and proliferation of hematopoietic cells (extramedullary hematopoiesis).	NTP, 1989[c]
				17 (F)		

Table 4-23. Subchronic, chronic, developmental, and reproductive oral toxicity studies for PCP

Species/strain	Dose (mg/kg-day)/ duration	Grade/type of PCP	NOAEL (mg/kg-day)[a]	LOAEL (mg/kg-day)[a]	Effect(s) at the LOAEL	Reference
Developmental/Reproductive						
Rat, Sprague-Dawley (15–20 pregnant dams/dose)	tPCP: 0, 5.8, 15, 34.7, or 50 aPCP: 0, 5, 15, 30, or 50 (gavage) GD 6–15	tPCP	5.8	15	Increased incidence of soft tissue and skeletal anomalies[b].	Schwetz et al., 1974a[c]
		aPCP	ND	5	Delayed ossification of the skull[b].	
Rat, Sprague-Dawley (15–20 pregnant dams/dose)	0, 10, 30, or 80 (gavage) GD 6–15	tPCP	30	80	Increased incidence of malformations[b] and skeletal variations[b], decreased live litter size and fetal body weight.	Bernard and Hoberman, 2001
Rat, Sprague-Dawley (10 M and 20 F/dose)	0, 3, or 30 (feed) 110 days, one-generation	EC-7	3	30	Decreased pup survival and growth, increased skeletal variations.	Schwetz et al., 1978
Rat, Sprague-Dawley (30/sex/dose)	0, 10, 30, or 60 (gavage) 110 days, two-generations	tPCP	ND	10	Delay in vaginal patency[b].	Bernard et al., 2002[c]
Rat, Sprague Dawley (20/sex/dose)	0, 4, 13, or 43 (feed) 181 days plus GD 1-20	aPCP	4	13	Increased skeletal variations[b], and dose-related decreases in fetal body weight and crown-rump length.	Welsh et al., 1987[c]

[a]M = male; F = female; ND = not determined.
[b]Denotes statistical significance.
[c]NOAELs and LOAELs determined by EPA for these studies; values for both genders unless otherwise specified.

A two-generation reproductive toxicity study in rats showed that exposure to tPCP is associated with decreased fertility, delayed puberty, testicular effects, decreased litter size, decreased viability, and decreased pup weights at a dose of 30 mg/kg-day (Bernard et al., 2002). These effects occurred at the same doses causing systemic toxicity in parental animals. A one-generation reproductive study in mink (1 mg/kg-day aPCP) showed evidence of reproductive effects in which many of the dams refused to accept the males for a second mating. Additionally, the whelping rate was reduced (Beard et al., 1997). However, a two-generation reproductive study of similar design reported no reproductive effects in mink administered 1 mg/kg-day PCP (Beard and Rawlings, 1998). Additionally, no effects on reproduction were noted in sheep (both ewes and rams) at a PCP dose of 1 mg/kg-day (Beard et al., 1999a, b).

The majority of developmental toxicity studies on PCP provided no evidence of teratogenic effects, but some older studies showed toxic effects of PCP in offspring that occurred at dose levels below those producing maternal toxicity. In Welsh et al. (1987), effects were observed in rat fetuses at 13 mg/kg-day compared with 43 mg/kg-day in the dams. Schwetz et al. (1974a) similarly reported sensitivity in fetuses at 5 mg/kg-day aPCP and 15 mg/kg-day tPCP compared with 30 mg/kg-day in the dams treated with either grade of PCP.

Studies show that treatment with PCP affected the levels of circulating thyroid hormones, T_3 and T_4. Serum T_3 and T_4 levels were significantly decreased by both aPCP and tPCP in rats (at a dose of 3 mg/kg-day, Jekat et al., 1994) and cattle (at a dose of 1 mg/kg-day, Hughes et al., 1985 and at a dose of 15 mg/kg-day, McConnell et al., 1980). Serum T_4 levels were significantly decreased by PCP (purity not reported) in ram and ewe lambs, and mink (at a of dose 1 mg/kg-day, Beard et al., 1999a, b; Beard and Rawlings, 1998), and by aPCP in mature ewes (at a dose of 2 mg/kg-day, Rawlings et al., 1998). PCP treatment did not affect the degree to which TSH stimulated thyroid hormone levels (Beard et al., 1999a, b). Only Jekat et al. (1994) reported changes in TSH levels following administration of PCP to rats for 28 days. Along with a decrease in T_4, there was a noted decrease in TSH. Because TSH levels were not elevated in response to the reduced thyroid hormone levels, the investigators concluded that PCP interfered with thyroid hormone regulation at the hypothalamic and pituitary levels. Additionally, the peripheral interference with thyroid hormone metabolism was suggested by the greater reduction in T_4 compared with T_3 (Jekat et al., 1994).

The mechanism by which PCP affects thyroid hormones has not been identified. Van den Berg (1990) reported that PCP competitively binds T_4 sites (i.e., for transthyretin, albumin, and thyroid binding globulin) and consequently induces inhibitory effects. Additionally, Den Besten et al. (1991) observed that PCP showed greater affinity for binding the T_4-binding site on thyretin (major T_4 transport protein) than T_4. The authors speculated that the binding to thyretin most likely resulted in the effects on thyroid homeostasis (Den Besten et al., 1991). Considering that similar effects were observed in rats and cattle with both tPCP and aPCP, the effect on serum thyroid hormone levels is attributed to PCP and not its impurities.

Studies examining the immunotoxic effects of PCP showed that the humoral response and complement activity in mice were impaired by tPCP, but not by aPCP, when administered to adult animals (at doses as low as 38 mg/kg-day [NTP, 1989]; 10 mg/kg-day [Holsapple et al., 1987; Kerkvliet et al., 1982a, b]; and 2 mg/kg-day [Kerkvliet et al., 1985a, b]). Treatment of mice with doses as low as 4 mg/kg-day from the time of conception to 13 weeks of age resulted in impaired humoral- and cell-mediated immunity (Exon and Koller, 1983). Blood measurements in humans with known exposure to PCP showed that immune response was impaired in patients who had blood PCP levels >10 μg/L and in particular in those whose levels were >20 μg/L (Daniel et al., 1995; McConnachie and Zahalsky, 1991).

In vitro neurotoxicity studies showed that 0.003–0.03 mM PCP causes a dose-dependent irreversible reduction in endplate potential at the neuromuscular junction and interference with axonal conduction in the sciatic nerve from the toad (Montoya and Quevedo, 1990; Montoya et al., 1988). An NTP (1989) study in mice showed decreased motor activity in rotarod performance in male rats treated with tPCP for 5 weeks and increases in motor activity and startle response in females receiving aPCP and tPCP for 26 weeks. Another in vivo study showed that treatment of rats with 20 mg/L PCP for up to 14 weeks caused biochemical effects in the rat brain (Savolainen and Pekari, 1979), although the authors considered these transient effects. The most definitive study showed that rats receiving 3 mM PCP in drinking water for at least 90 days had marked morphological changes in sciatic nerves (Villena et al., 1992). It is possible that some of the neurotoxic effects are related to PCP contaminants. Most of the neurotoxicity studies were performed using tPCP or PCP of unknown purity. NTP (1989) utilized four grades (aPCP, tPCP, DP-2, and EC-7) of PCP, ranging in dose from 36 to 458 mg/kg-day, and found that the majority of the neurotoxic effects were observed in male mice with tPCP; however, similar effects were also observed in female mice treated with all four grades of PCP. Effects were observed at the lower doses (36–102 mg/kg-day) and exhibited dose-related increases.

4.6.2. Inhalation

There are no human or animal data available to evaluate the consequences of long-term inhalation exposure to PCP. Toxicokinetic studies show that PCP is efficiently absorbed from the respiratory tract after single or repeated exposures and that a large portion of PCP is excreted in the urine as the unmetabolized parent compound with little evidence of binding in the tissues or plasma (Hoben et al., 1976a). In subchronic studies in rats and rabbits (Demidenko, 1969), minor liver function, cholinesterase activity, and blood sugar effects were reported in animals exposed to 2.97 mg/m^3 (calculated as 0.3 mg/kg-day PCP by Kunde and Böhme, [1978], a dose that is lower than the lowest NOAELs (1 mg/kg-day) observed in animals orally exposed to PCP. Demidenko (1969) reported anemia, leukocytosis, eosinophilia, hyperglycemia, and dystrophic processes in the liver of rats and rabbits exposed to 28.9 mg/m^3 PCP. Ning et al. (1984) reported

significant increases in organ weights (lung, liver, kidney, and adrenal glands), serum γ-globulin, and blood-glucose levels at 21.4 mg/m^3.

4.6.3. Mode-of-Action Information

Liver necrosis, chronic inflammation, hepatocellular vacuolation, pigmentation, and hepatic hypertrophy following chronic oral exposure to relatively low-doses (1.5–30 mg/kg-day) of PCP demonstrate that the liver is the target organ involved in PCP-induced toxicity. Liver necrosis was observed in subchronic (NTP, 1989; Kerkvliet et al., 1982b) and chronic-duration studies in mice (NTP, 1989), in subchronic-duration studies in rats (Villena et al., 1992; Johnson et al., 1973), and in a two-generation reproductive study in rats (Bernard et al., 2002). Chronic exposure to PCP induced inflammation in the liver of mice (NTP, 1989), rats (Bernard et al., 2002; NTP, 1999; Kimbrough and Linder, 1978; Schwetz et al., 1978), and dogs (Mecler, 1996), and in olfactory epithelium of rats (NTP, 1999). Additional evidence of lethal hepatocellular damage was reported by the majority of the studies in the database.

Oxidation/reduction processes have repeatedly been shown to be involved in PCP toxicity at doses of 60 mg/kg-day (NTP, 1999) and 25 μM (Dahlhaus et al., 1996, 1994). Dahlhaus et al. (1994) also observed oxidative stress at 300 mg/kg TCpHQ (metabolite of PCP) after 2 or 4 weeks of exposure. Damaged lipid membranes and induction of apoptosis (Wang et al., 2001) are some of the effects observed following exposure to 15 and 40 mg/kg PCP. The uncoupling of oxidative phosphorylation has long been associated with exposure to 0.25 μM to 1 mM PCP (Gravance et al., 2003; Wang et al., 2001; Trenti et al., 1986a, b; Varnbo et al., 1985; Masini et al., 1985, 1984a, b). The earliest detectable intracellular indication of an adverse redox shift is the appearance of lamellar aggregations of damaged lipid membranes (at the electron microscopy level), followed by uncoupling of oxidative phosphorylation and induction of apoptosis (Wang et al., 2001). PCP, as low as 0.1 mM, accelerated the breakdown of mitochondrial ATP, a likely consequence of changed membrane permeability (Weinbach, 1954). PCP was noted as inhibiting the electron transport between flavin coenzyme and CYP450 (which may explain the limited metabolism associated with PCP). Thus, PCP was recognized as capable of interacting with, and interfering with, multiple molecular intracellular target molecules and cellular processes. The inhibition of oxidative phosphorylation, at 40 mg/kg, has been suggested to precede heptatocellular necrosis (Arrhenius et al., 1977a). Increased cellular phospholipoperoxides and greatly decreased glutathione have been observed following incubation with 1 mM PCP (Suzuki et al., 2001). Antioxidant protective systems can become overwhelmed in the presence of intracellular redox disruption. Depletion of glutathione combined with the potential for oxidative damage suggests that PCP can induce nonneoplastic effects in multiple animal species.

4.6.4. Comparison of Toxic Effects of Analytical PCP with Technical or Commercial Grades of PCP

PCP is manufactured in a multistage chlorination process that results in contamination with dioxins, furans, and other chlorophenols. Consequently, the formulation that is employed and that people are exposed to is a chemical grade that has a purity of approximately 90%, and is commonly referred to as the technical or commercial grade of PCP. Depending on the specific synthesis process, the level of these impurities may vary with differing grades of manufactured PCP. Analytical-grade PCP is only achieved after the impurities are removed. Therefore, the information available on toxic effects from PCP alone is limited. There are studies in the database that have examined the toxicity of aPCP, either alone or concurrently with the technical/commercial grades (tPCP, EC-7, and/or DP-2). The toxicity database for PCP contains many studies that did not characterize the type and/or level of the contaminants. The uncertainty surrounding the presence of these contaminants confounds the characterization of PCP itself. However, a comparison of toxicity studies conducted with the analytical grade (>99% purity) with studies using commercial preparations is useful.

4.6.4.1. *Short-term and Subchronic Studies*

In a subchronic study, rats exhibited increased liver weight at doses of 10 and 30 mg/kg-day and increased kidney weight at 30 mg/kg-day (Johnson et al., 1973, 90-day feed study) with both aPCP and an "improved" grade (88–93% purity) of PCP. tPCP administration elicited elevated liver and kidney weight at 3, 10, and 30 mg/kg-day. Additionally, at a dose level of 30 mg/kg-day tPCP, serum albumin and hepatic microscopic lesions (minimal focal hepatocellular degeneration and necrosis) were elevated and erythrocyte count, hemoglobin concentration, and hematocrit were reduced. For aPCP, Renner et al. (1987) reported decreased erythrocyte parameters (RBC, hemoglobin, and hematocrit) throughout 4 weeks of treatment (53 mg/kg-day) via gavage. Liver effects, including enlarged pleomorphic hepatocytes, degeneration of liver cells, and acidophilic bodies in sinusoids, were observed in addition to the hematological effects. The hepatic and hematological effects observed with 30 mg/kg-day tPCP and not aPCP in Johnson et al. (1973) were seen with aPCP at a concentration of 53 mg/kg-day in Renner et al. (1987). In an NTP (1999) study, hepatocyte degeneration increased in incidence and severity at aPCP doses of 40 and 75 mg/kg-day in male and female rats, respectively. Degeneration of germinal epithelium in testes in males and centrilobular hypertrophy in males and females were observed at 270 mg/kg-day aPCP (highest dose) (NTP, 1999, 28-day study).

Kimbrough and Linder (1975) reported cytoplasmic inclusions and ultrastructural effects (increased smooth endoplasmic reticulum, presence of lipid vacuoles, and atypical appearance of mitochondria) at 1,000 ppm (approximately 87 mg/kg-day) of either tPCP or aPCP for 90 days. In addition, tPCP-treated animals exhibited hepatic effects consisting of foamy cytoplasm, pronounced vacuolation of hepatocytes, single hepatocellular necrosis, slight interstitial fibrosis,

and prominent brown pigment in macrophages and Kupffer cells in liver. In Kimbrough and Linder (1978), rats administered tPCP and aPCP for 8 months showed signs of liver toxicity at 500 ppm (approximately 46 mg/kg-day), including cytoplasmic hyaline inclusions, hepatocellular hypertrophy, and abundant brown pigment in macrophages and Kupffer cells. As in the 1975 study, additional liver effects were observed in those animals treated with tPCP (periportal fibrosis, adenofibrosis, vacuolation, pleomorphism, and bile duct proliferation). Hepatic effects were also observed at 10 mg/kg-day, although these effects were limited to animals treated with tPCP.

NTP (1989) noted liver lesions consisting of centrilobular cytomegaly, karyomegaly, nuclear atypia, and degeneration, and necrosis in male mice treated for 30 days with 500 ppm (95 mg/kg-day for males and 126 mg/kg-day for females) tPCP, EC-7, and aPCP. Female mice showed signs of liver toxicity with EC-7 and aPCP at doses of 645 and 25 mg/kg-day, respectively. The report stated that hepatic lesions in animals treated with EC-7 and aPCP were less diffuse and less severe than with tPCP. However, the incidences of the lesions were similar for tPCP and aPCP for all doses. All grades of PCP exhibited increases in absolute and relative liver weights, liver porphyrins, P450 levels, and serum enzymes (ALP, cholesterol, and ALT), and a decrease in leukocyte count (males only).

In a 27-week study (NTP, 1989), mice treated with tPCP, EC-7, DP-2, and aPCP showed results similar to the 30-day study. Hepatic cytomegaly, karyomegaly, degeneration, and necrosis were observed in males and females at all doses (estimated average doses are 36–458 mg/kg-day) and grades of PCP. While all four grades elicited effects at the high dose, including liver pigmentation, liver inflammation, dark urine, and urine creatinine, only tPCP showed signs of bile duct hyperplasia. Liver pigments were seen at the low and mid dose for tPCP and at the mid dose for DP-2 and EC-7. aPCP-treated animals did not show signs of liver pigmentation, inflammation, or urinary effects at doses other than the high dose. Similar hepatotoxic effects were shown for aPCP and tPCP, including mild to marked hepatocyte swelling, and increases in relative liver weight, nuclear swelling, vacuolization with eosinophilic inclusions in nuclear vacuoles, and mild to moderate multifocal necrosis in the liver (Kerkvliet, 1982a, b).

tPCP was observed to have significantly higher levels of chlorinated dibenzo-p-dioxins and dibenzofurans than either DP-2 or EC-7. Specifically, the concentration of heptachlorodibenzo-p-dioxin was observed to be approximately 10 and 500 times higher for tPCP than for DP-2 and EC-7, respectively. Higher concentrations were also observed for OCDD and HxCDD. Thus, mice were exposed to higher levels of these contaminants from tPCP-treated feed than from DP-2- or EC-7-treated feed (NTP, 1989). Despite this, there were no differences in liver toxicity caused by tPCP and EC-7, suggesting that PCP, itself, causes liver toxicity in the mice. Only tPCP resulted in significant increases in the incidences of lesions in the spleen of male mice and mammary gland of female mice, suggesting that these lesions were

caused by impurities. Lesions in the nose were prominent in mice receiving EC-7 but not in mice receiving tPCP, suggesting that a specific EC-7 impurity (possibly TCP which is present in greater amounts in EC-7 compared with tPCP) caused these lesions.

Dose-dependent decreases in motor activity and rotarod performance were found in mice treated with tPCP only. Immunosuppression in the form of inhibition of plaque-forming response following immunization with SRBCs was seen at all doses of tPCP and at the highest dose of DP-2 and not observed with EC-7 or aPCP. NTP (1989) stated that the degree of immunosuppression is consistent with exposure to dioxin and furan contamination. Studies in Swiss Webster, C57BL/6J, and DBA/2J mice showed immunosuppressive effects in animals treated with tPCP but not with aPCP (Kerkvliet 1985a, b; 1982a, b). In an experiment looking at tPCP only, mice exhibited a significant increase in relative liver weight as well as effects on humoral but not cellular immunity (Kerkvliet, 1985b). The remaining studies observed differences in effects from treatment with aPCP and tPCP. Significant depression of T-lymphocyte cytolytic activity and enhancement of macrophage phagocytosis (Kerkvliet, 1982b) as well as early immunosuppressive effects on humoral response (Kerkvliet, 1982a) were observed with tPCP treatment and no effects were seen with aPCP, even at doses fourfold greater than tPCP doses. Additionally, contaminant fractions from tPCP, at equivalent doses to tPCP, were examined for immunotoxic effects. The chlorinated dioxin/furan fraction had a significant immunosuppressive effect, whereas the chlorinated phenoxyphenol and the chlorinated diphenyl ether fractions were ineffective in affecting the immune response (Kerkvliet, 1985a). These studies show that the chlorinated dioxin and furan contaminants present in tPCP and not PCP are likely responsible for the immunotoxic effects observed in mice. However, Exon and Koller (1983) reported a significant depression in immune response (humoral and cell-mediated immunity) in offspring of male and female Sprague-Dawley rats administered 4 or 43 mg/kg-day and 5 or 49 mg/kg-day aPCP, respectively, continuously in the diet from weaning until 3 weeks after parturition. Offspring were treated similarly to the parents and treatment continued until 13 weeks of age. Macrophage function measured by the rats' ability to phagocytize SRBCs increased in a dose-related manner that was statistically significant at 4 and 43 mg/kg-day for males and 5 and 49 mg/kg-day for females. In addition, there was an increase in the number of macrophages harvested from the peritoneal exudate.

In cattle, aPCP caused significant decreases in serum T_3 and T_4 levels at 10 (Hughes et al., 1985) and 15 mg/kg-day (McConnell et al., 1980). However, tPCP-treated animals also exhibited microscopic lesions consistent with thymus atrophy, squamous metaplasia in the Meibomian gland of the eyelid (Hughes et al., 1985; McConnell et al., 1980), and smaller and more numerous thyroid-follicles (McConnell et al., 1980). McConnell et al. (1980) attributed the dose-related effects that were observed with tPCP and not aPCP to the dioxin and furan contaminants in tPCP. Jekat et al. (1994) reported decreases in total and free serum T_4, T_4:T_3 ratio in serum, and serum TSH in female Wistar rats administered 3 mg/kg-day aPCP or tPCP by

gavage for 28 days. In a two-generation study in mink exposed to 1 mg/kg-day PCP, Beard and Rawlings (1998) reported statistically significant decreases in serum T_4 secretion in the F1 (21%) and F2 (18%) males and F2 females (17%). Thyroid mass was decreased in both F1 and F2 generation animals, although reduction was statistically significant only in F2 females (27%). Rawlings et al. (1998) administered 2 mg/kg aPCP to mature ewes for approximately 6 weeks. A marked decrease in serum T_4 levels was observed in mature ewes at 36 days. In addition to statistically significant decreased serum T_4 levels, aPCP-treated ewes had significantly increased serum insulin levels. However, no treatment-related changes were observed in cortisol, LH, FSH, estradiol, or progesterone levels. Beard et al. (1999a) noted maximum serum T_4 levels in 1 mg/kg-day PCP-treated ewes were statistically significantly lower (approximately 25%) than controls with or without prior administration of TSH.

4.6.4.2. *Chronic Studies*

Within the PCP database, only one study examined the effects of chronic exposure to aPCP. NTP (1999) reported significantly increased cystic degeneration of hepatocytes in male rats at 20 and 30 mg/kg-day in a 2-year bioassay. However, in an additional stop-exposure portion of this study, rats administered 60 mg/kg-day for 1 year exhibited significantly elevated serum ALP and cytoplasmic hepatocyte vacuolization in males, increased sorbitol dehydrogenase, and incidences of centrilobular hypertrophy in both males and females. ALT levels were elevated in male rats, although this increase was not considered statistically significant. In another chronic study in rats, Schwetz et al. (1978) reported slightly increased (<1.7-fold) serum ALT activity in both sexes at 30 mg/kg-day EC-7.

Additionally, rats treated with 60 mg/kg-day aPCP (NTP, 1999) exhibited liver lesions including chronic inflammation, basophilic focus, and cystic degeneration of hepatocytes. Renal tubule pigmentation was observed in all rats of this study at doses ranging from 10 to 60 mg/kg-day (2-year bioassay and 1-year stop-exposure). Analyses of the pigment were inconclusive as a result of contrasting staining results. Histopathological examination in Schwetz et al. (1978) showed pigment accumulation in the centrilobular hepatocytes of the liver in 30% of females given 10 mg/kg-day and in 59% of females given 30 mg/kg-day. Similarly, 26 and 70% of females receiving 10 and 30 mg/kg-day EC-7 exhibited pigment accumulation in the epithelial cells of the proximal convoluted tubules in the kidney. This effect was not detected in the lower dose or control groups of the female rats. Only one of the 27 male rats given EC-7 (30 mg/kg-day) exhibited the brown pigment in hepatocytes. NTP (1989) reported hepatotoxic effects in mice at doses as low as 17 mg/kg-day EC-7 or tPCP that are similar to those reported in rats ranging from 10 to 60 mg/kg-day reported by NTP (1999) [aPCP] and Schwetz et al. (1978) [EC-7].

4.6.4.3. *Developmental Studies*

Schwetz et al. (1974a) examined the maternal and fetal effects of rats administered tPCP or aPCP on GDs 6–15. Similar effects were observed for both grades of PCP, including significant decreases in maternal and fetal weight gain at 30 and 50 mg/kg-day. A statistically significant increased incidence of resorptions was noted at 15 mg/kg-day for tPCP and 30 mg/kg-day for aPCP. While tPCP did not seem to affect fetal crown-rump length, aPCP-treated rats exhibited significantly decreased crown- rump length at 30 mg/kg-day. Soft-tissue and skeletal anomalies were induced with doses ≥15 mg/kg-day tPCP and ≥5 mg/kg-day aPCP. In a timing evaluation of PCP administration, significant decreases in fetal body weight and crown-rump length and increased incidence of subcutaneous edema and rib, vertebral, and sternebral anomalies were observed following administration of 30 mg/kg-day PCP on GDs 8–11 for tPCP and aPCP and on GDs 12–15 for aPCP only. The authors stated that aPCP exhibited greater toxicity than tPCP, especially in the latter stage of gestation. The effects observed in the developing rat embryo and fetus were attributed to PCP and not the contaminants (Schwetz et al., 1974a).

Developmental toxicity was noted at a dose level of 60 mg/kg-day in the Larsen et al. (1975) study in which rats exposed to aPCP during gestation had fetuses with reduced body weight and increased malformations. The authors concluded that the maternal toxicity resulted in the observed fetal effects. This was based on other study findings indicating limited transfer of PCP through the placental barrier. However, Larsen et al. (1975) did not report the maternal toxicity data. Welsh et al. (1987) also observed fetal effects following administration of aPCP at doses of 13 and 43 mg/kg-day. Significantly decreased body weight and crown-rump length and increased skeletal variation (misshaped centra) were observed in fetuses at 13 and 43 mg/kg-day. The dams exhibited signs of toxicity, such as decreased mean weight gain (GDs 7–20) and decreased number of viable fetuses, because of significant resorption at the 43 mg/kg-day dose level.

Summary of comparison of toxic effects of analytical PCP with technical/commercial PCP. Repeated dose toxicity studies with tPCP, EC-7, DP-2, and/or aPCP formulations all show the liver to be a major target. Many of the studies comparing tPCP and aPCP showed similar toxic effects following exposure to each formulation. Studies that compared toxicity of purified and technical grade PCP show a broader spectrum of liver toxicity occurring at similar or slightly lower doses with tPCP than aPCP (NTP, 1989; Hughes et al., 1985; McConnell et al., 1980; Kimbrough and Linder, 1978; Johnson et al., 1973). Therefore, EPA determined that studies using technical or commercial grades of PCP are representative of PCP itself, and that an RfD based on these studies should also apply to pure PCP.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), PCP is "likely to be carcinogenic to humans." This cancer weight of evidence determination is based on (1) evidence of carcinogenicity from oral studies in male mice exhibiting hepatocellular adenomas and carcinomas, pheochromocytomas and malignant pheochromocytomas, and in female mice exhibiting hepatocellular adenomas and carcinomas, pheochromocytomas and malignant pheochromocytomas, and hemangiomas and hemangiosarcomas (NTP, 1989); (2) some evidence of carcinogenicity from oral studies in male rats exhibiting malignant mesotheliomas and nasal squamous cell carcinomas (Chhabra et al., 1999; NTP, 1999); (3) strong evidence from human epidemiologic studies showing increased risks of non-Hodgkin's lymphoma and multiple myeloma, some evidence of soft tissue sarcoma, and limited evidence of liver cancer associated with PCP exposure (Demers et al., 2006; Hardell et al., 1995, 1994; Kogevinas et al., 1995); and (4) positive evidence of hepatocellular tumor-promoting activity (Umemura et al., 2003a, b, 1999) and lymphoma and skin-adenoma promoting activity in mice (Chang et al., 2003).

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the cancer descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Oral studies of PCP carcinogenicity demonstrate that tumors occur in tissues remote from the site of absorption, including the liver, adrenal gland, circulatory system, and nose. Information on the carcinogenicity of PCP via the inhalation and dermal routes is unavailable. Studies of the absorption of PCP indicate that the chemical is readily absorbed via all routes of exposure, including oral, inhalation, and dermal. Therefore, based on the observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Accordingly, PCP is considered "likely to be carcinogenic to humans" by all routes of exposure.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

4.7.2.1. *Human Epidemiologic Evidence*

Epidemiological studies of various designs (cohort, population-based case-control, and nested case-control within occupationally exposed workers) have examined the relationship between occupational PCP exposure and cancer risk. The most comprehensive of the cohort studies, in terms of design, is the sawmill cohort study conducted in British Columbia, Canada, recently updated by Demers et al. (2006). In addition to the sample size, the design of this study including the exposure assessment procedure; use of an internal referent group; analysis of PCP and TCP exposures; low loss to follow-up; and use of a population-based cancer registry add to

the strengths of this study. Even with this size, however, there is limited statistical power to estimate precise associations with relatively rare cancers. The case-control studies of non-Hodgkin's lymphoma and soft tissue sarcoma (Hardell and Eriksson, 1999; Kogevinas et al., 1995; Hardell et al., 1995, 1994) specifically address this limitation by focusing on these outcomes. Kogevinas et al. (1995) has the additional attribute of providing estimates for the effects of other phenoxy herbicides or chlorophenols, which provides information regarding the issue of co-exposures.

In these studies, moderately high associations (i.e., a two- to fourfold increased risk) were generally seen between occupational exposure to PCP and non-Hodgkin's lymphoma (Demers et al., 2006; Kogevinas et al., 1995; Hardell et al., 1994), multiple myeloma (Demers et al., 2006), or soft tissue sarcoma (four studies summarized in a meta-analysis by Hardell et al., 1994). However, there are some inconsistencies, most notably for soft tissue sarcoma. The relative rarity of this cancer (e.g., only 12 cases were found in the nested case-control study of 13,898; workers exposed to phenoxy herbicides or chlorophenols by Kogevinas et al. [1995]), and difficulty in classifying the disease, even with a review of the histology, may be reasons for this inconsistency. In contrast to the studies from the 1970s and 1980s, the most recent case-control study of non-Hodgkin's lymphoma, conducted in cases diagnosed 9–13 years after PCP had been banned from use in Sweden, did not observe an association (OR 1.2) with PCP exposure (Hardell and Eriksson, 1999). The lack of association in this study could reflect a relatively short latency period between exposure and disease, as has been seen with other lymphoma-inducing agents (e.g., Krishnan and Morgan, 2007).

Demers et al. (2006) developed a cumulative dermal chlorophenol exposure score based on a retrospective exposure assessment that was validated for current exposures in comparison with urinary measurements and with industrial hygienist assessments. This detailed exposure measure allowed for analysis of an exposure-response gradient, with evidence of a trend of increasing mortality or incidence risk seen for non-Hodgkin's lymphoma and multiple myeloma. The other studies with a relatively detailed exposure assessment (Hardell et al., 1995, 1994; Kogevinas et al., 1995) also demonstrated stronger associations with the more refined (e.g., higher exposure probability or frequency) measures of exposure compared with the associations seen with "any pentachlorophenols."

The possibility of the carcinogenic effects of PCP resulting solely from the presence of contaminants of dioxins and furans was examined in this assessment. The primary contaminants are hexa-, hepta-, and octa-chlorinated dibenzodioxins, and higher-chlorinated dibenzofurans. There are several reasons, as noted in Section 4.1.1.4 (General Issues—Interpretation of the Epidemiologic Studies) that this contamination is an unlikely explanation for the observed effects. Specific furans are not generally seen at higher levels in blood from PCP workers compared with the general population (Collins et al., 2007). The cancer risks seen in the large cohorts of workers exposed to dioxins (consistent observations of an exposure-response gradient

with total cancer risk) (NAS, 2006; Steenland et al., 2004) differ from the observations seen in studies of PCP exposure. In addition, the associations seen with specific cancers (e.g., non-Hodgkin's lymphoma) and PCP are generally stronger than the associations seen between these cancers and dioxin or other chlorophenol exposures in studies with both of these measures (Demers et al., 2006; Kogenivas et al., 1995).

An increased risk of liver cancer associated with exposure to PCP was seen in the large cohort study of sawmill workers in British Columbia (Demers et al., 2006), and as noted in the previous discussion of non-Hodgkin's lymphoma, an attenuation in the highest exposure group was observed. This study identified strong associations between exposure to PCP and liver cancer, with at least a doubling of the risk in almost all of the exposure categories.

Evidence for PCP-induced DNA damage has been presented in numerous animal or in vitro studies and was equivocal in studies of PCP-exposed workers (Ziemsen et al., 1987; Bauchinger et al., 1982; Schmid et al., 1982). Evidence for cytotoxicity or apoptosis, reparative cell proliferation, and gap junction inhibition usually cannot be obtained in human studies.

PCP-induced effects on the immune system have been found in humans and animals. Blakley et al. (1998) reported stimulation of mitogen effects in low-dose, gavage-treated male rats. Daniel et al. (1995) observed exposure-dependent impairment of mitogen response in lymphocytes of PCP-exposed humans, and McConnachie and Zahalsky (1991) reported heightened immune response in PCP-exposed humans. Finally, symptoms of porphyria were identified in PCP-exposed humans (Cheng et al., 1993) and animals (NTP, 1989; Kimbrough and Linder, 1978). These findings make a strong point for the plausibility of PCP-related carcinogenesis in humans. In summary, the weight of evidence for the carcinogenic action of PCP (U.S. EPA, 2005a) suggests that this compound by itself (i.e., in the absence of contaminants) is likely to be a human carcinogen.

4.7.2.2. *Animal Cancer Evidence from Oral Exposure*

Long-term animal studies employing the oral route of exposure are available that assess the carcinogenicity of PCP in animals. An NTP feeding study in $B6C3F_1$ mice demonstrated that tPCP (17–18 or 35–36 mg/kg-day) and EC-7 (17–18, 35–36, or 117–118 mg/kg-day) caused statistically significant increases in the incidence of hepatocellular adenomas/carcinomas and adrenal gland pheochromocytomas in males and females, and an increased incidence of hemangioma/hemangiosarcoma in female mice (NTP, 1989). tPCP was slightly more effective than EC-7, suggesting that chlorinated dibenzo-p-dioxin and dibenzofuran impurities in tPCP may have only exacerbated the carcinogenic effect of PCP in mice.

Another NTP (1999) feeding study conducted in F344/N rats provided some evidence of carcinogenic activity, demonstrated by increased incidence of mesotheliomas and nasal squamous cell carcinomas in males exposed to aPCP (10–60 mg/kg-day). NTP (1999) concluded that there was no evidence of carcinogenic activity for female rats fed aPCP.

Umemura et al. (1999) examined the initiating and promoting activity of aPCP (98.6% purity) administered in the diet to 20 male B6C3F1 mice/group. Diethylnitrosamine (DEN) was given as the initiator when the promoting activity of aPCP was assessed, and PB was administered as the promoter when the initiating activity of aPCP was assessed. The incidence of liver tumors was statistically significantly higher in mice initiated with DEN and promoted with PCP than in control mice receiving DEN only. Tumor multiplicity was statistically significantly increased in mice promoted with aPCP and PB compared with DEN controls. No liver tumors developed in mice initiated with aPCP with or without subsequent promotion with PB. In this study, aPCP showed promoting, but not initiating, activity in mice that were initiated with DEN. Umemura et al. (1999) concluded that aPCP exerts a promoting effect on liver carcinogenesis.

A study by Bionetics Research Laboratories, Inc. (BRL, 1968) showed no carcinogenic response in male and female $B6C3F_1$ and B6AKF1 mice administered EC-7 at a dose of 46.4 mg/kg-day for up to 18 months. This exposure may not have been long enough to reveal carcinogenic effects. BRL (1968) also reported that mice administered 46.4 mg/kg-day EC-7 as a single, subcutaneous injection did not develop tumors that were considered statistically significantly greater than tumors observed in control animals. Schwetz et al. (1978) reported no carcinogenic response in male and female Sprague-Dawley rats administered EC-7 in the diet at doses up to 30 mg/kg-day for 22–24 months. A lack of body or organ weight changes even at the highest dose raise the possibility that an MTD was not reached in this study.

Potential toxicity of contaminants.

The potential carcinogenicity of the contaminants associated with PCP was considered when assessing the carcinogenicity associated with exposure to PCP. NTP (1989) listed an estimate of the total contaminant exposure associated with tPCP and EC-7 in the mouse 2-year bioassay. Most importantly, the most potent carcinogenic promoter ever studied (Pitot et al., 1980), TCDD, has not been detected in the PCP preparations. Contaminant levels increased with the degree of chlorination; the highest levels were detected for OCDD (400 and 800 µg from tPCP, or 0.2, 0.4, and 1.2 µg from EC-7). Total exposure to pentachlorodibenzofuran was estimated at approximately 0.01–0.03 µg/kg-day for tPCP at the 17–18 and 35–36 mg/kg-day doses over the full 2-year period. This compound was not detected in EC-7. Additional contaminants identified at comparatively high levels in tPCP were octachlorohydroxydiphenyl ether (0.2–0.4 mg/kg-day), nonachlorohydroxydiphenyl ether (0.4–0.8 mg/kg-day), hexachlorohydroxydibenzofuran (0.02–0.04 mg/kg-day), and heptachlorohydroxydibenzofuran (0.05–0.1 mg/kg-day). These ether contaminants were not detected in EC-7. A complete list of the contaminants can be found in Table 2-1 and estimated daily doses can be found in Table B-3.

NTP (1989) and McConnell et al. (1991) compared the concentrations of HxCDD in tPCP and EC-7 with that known to induce liver tumors in mice and concluded that the

carcinogenic response in mice can be attributed primarily to PCP. Hepta- and octachlorodibenzo-p-dioxins and dibenzofurans, because of their very poor bioavailability and metabolism, have comparatively low toxicity. Toxicity data for the higher chlorinated hydroxydibenzofurans or hydroxydiphenyl ethers are not available.

The major contaminant measured in both formulations of PCP utilized by NTP (1989) was TCP, present at levels yielding doses of 0.4–0.9 mg/kg-day in tPCP at the 17–36 mg/kg-day doses and 1.0–6.0 mg/kg-day in EC-7 at the 17–118 mg/kg-day doses, respectively. In the absence of a slope factor for any of the TCP congeners, the possible contribution of this contaminant to the carcinogenicity of tPCP or EC-7 cannot be determined. However, considering the difference in the amount of TCP that was found in tPCP versus EC-7 compared to the similar tumor responses observed for the two formulations, a reasonable assumption would be that, at the given doses, the contribution of TCP to the carcinogenicity of tPCP or EC-7 is likely to be minimal.

4.7.2.3. *Animal Cancer Evidence from Inhalation Exposure*

There are no known chronic duration inhalation exposure studies in humans or laboratory animals. Limited evidence concerning the potential effects induced by PCP inhalation is based on evidence of respiratory tract effects in three animal studies. In the NTP (1999) stop-exposure oral study of F344/N rats showing nasal squamous cell carcinomas in males, Chhabra et al. (1999) suggested that the cancers were chemical related, either via systemic exposure, via direct nasal contact with PCP vapors during feeding, or via PCP-containing feed dust. In an earlier NTP (1989) study, increased incidences of acute focal inflammation of the nasal mucosa (males: 4/35, 1/13, 3/16, 47/49; females: 0/35, 0/14, 2/5, 46/48) and focal metaplasia of the olfactory epithelium (males: 2/35, 1/13, 2/16, 46/49; females: 1/35, 0/14, 2/5, 45/48) were observed in mice that received EC-7 in feed (at doses of 0, 17–18, 34–37, and 114–118 mg/kg-day, respectively) but not in mice exposed to tPCP (NTP, 1989).

NTP (1989) conducted a 6-month range-finding study in $B6C3F_1$ mice fed four different preparations of PCP (tPCP, DP-2, EC-7, and aPCP). Increased incidences of nasal mucosal metaplasia/goblet cell hyperplasia were seen in female mice that received doses of 54 or 51 mg/kg-day EC-7 or aPCP, respectively, or 323 mg/kg-day DP-2 and in male mice that received doses of 124 mg/kg-day EC-7 or 102 mg/kg-day aPCP. Mice, both male and female, administered tPCP (38–301 mg/kg-day) did not show any of the nasal effects. Females were more sensitive to the nasal effects than male mice.

Tisch et al. (2005) obtained evidence for single and double strand breaks in ex vivo cultures of human mucosal cells of the inferior and middle nasal conchae treated with 0.3, 0.75, and 1.2 mmol/mL aPCP. According to the authors of the study, as much as 1.5 mmol PCP has been measured in nasal mucosa in the presence of dust contaminated with PCP in occupational inhalation studies. These results indicate that humans may be exposed to concentrations of PCP

that have induced DNA damage in human mucosal cells, although Tisch et al. (2005) observed the damage in cells that lacked a protective mucosal barrier normally present in humans in vivo. While many of the human epidemiological studies (Kogevinas et al., 1992; Saracci et al., 1991; Brinton et al., 1977) suggest an inhalation cancer risk the lack of useable exposure levels, possible presence of contaminants and other study limitations prevent clear associations between PCP exposure and cancer in these reports.

4.7.3. Mode-of-Action Information

PCP can interact directly via parent compound or indirectly via metabolites with cellular biomolecules, including lipids, proteins, and nucleotides. PCP has not shown strong mutagenic activity in standard genotoxicity tests such as the Ames assay (Seiler, 1991). Positive results have been observed for PCP in tests that respond to molecular action other than direct mutation, such as SCE induction; however, PCP-induced SCEs could not be confirmed in exposed humans (Ziemsen et al., 1987; Bauchinger et al., 1982; Schmid et al., 1982). SSBs and CAs were observed in animals and exposed humans in assays using PCP or TCHQ. The metabolites of PCP, specifically TCHQ, TCoHQ, TCpBQ, and TCpCAT, have shown some evidence of SSBs in in vitro assays. TCpHQ was positive for forward mutations in V79 Chinese hamster cells at the HPRT locus (Jansson and Jansson, 1991). Carstens et al. (1990) suggested that superoxide formation with TCHQ and reduction of H_2O_2 by TCSQ (in the Fenton reaction) may result in cellular toxicity and genotoxicity. However, PCP is rather poorly metabolized in animals (see Section 3.1) and to what extent the metabolites are formed is unknown. Without more information on the formation of the metabolites, it is difficult to determine the influence that the parent compound or the metabolites have on mutagenic activity.

While standard mutagenicity assays have produced weak or equivocal evidence for PCP, there is some in vitro and in vivo evidence for the ability of PCP to cause oxidative DNA damage. Several studies presented evidence that long-term administration of PCP results in measurable 8-OH-dG formation in hepatic nuclear DNA of mice (Umemura et al., 1996; Sai-Kato et al., 1995) and rats (Lin et al., 2002). Naito et al. (1994) demonstrated that PCP induced DNA damage via 8-OH-dG formation through its metabolite, TCHQ, in calf thymus DNA in vitro. Dahlhaus et al. (1994) showed that TCpHQ elicited increased 8-OH-dG formation in hepatic DNA of $B6C3F_1$ mice fed this PCP metabolite for 2 or 4 weeks, while single i.p. injections had no such effect. Dahlhaus et al. (1996, 1995) found that TCpHQ, TCpBQ, and TCoBQ produced 8-OH-dG, while TCoHQ and PCP did not. Formation of 8-OH-dG was specific for the liver, the target organ. Significant decreases in the levels of glutathione, a protective antioxidant, were observed following exposure to PCP (Suzuki et al., 2001, 1997; Savolainen and Pekari, 1979) and TCHQ (Wang et al., 1997).

In addition to oxidative stress-induced DNA damage, the formation of DNA adducts by metabolites of PCP has been observed in both in vitro and in vivo studies. TCpBQ was frequently identified as the major metabolite responsible for the formation of the DNA and protein adducts associated with PCP exposure. Studies have shown that dechlorination of PCP to the 1,4-chlorinated benzoquinone resulted in increases of DNA adducts in vitro at 100 μM (Dai et al., 2005, 2003) and at 1 or 5 mM (Lin et al., 2001) and in vivo (Lin et al., 2002; Bodell and Pathak, 1998). Rats exhibited DNA adducts following administration of PCP, TCHQ, and TCpBQ. Typically, PCP and TCHQ are oxidized to facilitate the formation of the benzoquinone radical, which is believed to be the reactive intermediate in the adduct formation (Lin et al., 2002). Additionally, protein adducts in albumin and hemoglobin were observed in rats exposed to TCpBQ, TCpSQ, and TCoSQ, but not TCoBQ (Waidyanatha et al., 1996), providing further evidence of oxidative stress induced DNA damage. Oxidative stress-induced DNA damage that occurs in concert with the formation of chemical-specific DNA adducts may enhance the genotoxic effects of PCP.

Lin et al. (1999) suggested that species differences in the metabolism of PCP to semiquinone and quinone metabolites may be responsible for the observed species differences in liver carcinogenicity (i.e., PCP induced liver tumors in mice but not rats). At low PCP doses (<4–10 mg/kg), TCoSQ-protein adduct formation in liver cytosol and nuclei was higher in rats than in mice. At high PCP doses (>60–230 mg/kg), however, TCpBQ adducts were higher in mice than in rats. Moreover, there was a fourfold difference in the nuclear total of quinone metabolites in the mouse compared with that in the rat (Lin et al., 1997). Lin et al. (1999) speculated that such differences in the metabolism of PCP to semiquinones and quinones might be responsible for the production of liver tumors in mice but not rats. This is supported by the results in Dahlhaus et al. (1996, 1995) in which TCpHQ and TCpBQ, but not TCoHQ, induced the formation of 8-OH-dG.

Various isozymes of P450 are responsible for metabolism of PCP and these may differ between the two rodent species. Specific enzyme induction in mice (eightfold increase versus control) versus the rat (2.4-fold increase versus control) may also be involved in the different tumor patterns for these animals (Mehmood et al., 1996; Van Ommen et al., 1986a). PCP-DNA adducts have been found at much higher amounts in mouse liver (Bodell and Pathak, 1998), possibly a consequence of higher amounts of PCP quinone metabolites found in mouse liver as compared with rat liver (Lin et al., 1997). Evidence of varied oxidative stress-generated quinone-DNA adducts in rats and mice administered PCP (La et al., 1998b) combined with the production of superoxide anion radical by mice, more so than other species (Parke and Ioannides, 1990) suggests species differences in the PCP-induced effects. These differences may explain the distinctive tumor patterns in mice and rats. Additionally, the findings concerning species differences in liver carcinogenicity of PCP were corroborated in other studies in which PCP

induced hepatocellular karyomegaly, cytomegaly, and degeneration in mice but only mild hepatotoxicity in exposed rats (NTP, 1989; Kimbrough and Linder, 1978).

A number of studies have shown that PCP causes not only oxidative DNA damage but also oxidative damage to other subcellular systems, specifically cellular membranes (Suzuki et al., 1997; Wang et al., 1997; NTP, 1989). It is well known that these events disrupt electron transport and metabolic energy synthesis (Freire et al., 2005; Masini et al., 1985; Arrhenius et al., 1977b; Weinbach, 1954), thereby contributing to cell death. Suzuki et al. (1997) reported a fivefold increase in cellular phospholipid hydroperoxide levels that were induced by PCP, while cellular glutathione was virtually eliminated by PCP treatment. The latter effect is a potentially critical event for PCP, allowing for oxidative stress to damage membranes, proteins, and nucleotides. Wang et al. (1997) reported depletion of glutathione by TCHQ. These results suggest that oxidative damage to cellular membrane phospholipids may be responsible for the cytotoxicity induced by PCP.

Several responses to PCP exposure—including necrosis and chronic inflammation leading to reparative cell proliferation/regeneration, and interference with GJIC—are consistent with a promoting effect of PCP. Liver cell necrosis, the prerequisite for reparative cell proliferation, has been observed in many experimental settings involving PCP exposure. Liver necrosis was observed in subchronic (NTP, 1989; Kerkvliet et al., 1982b) and chronic (NTP, 1989) duration studies in mice, in subchronic (Villena et al., 1992; Kimbrough and Linder, 1975; Johnson et al., 1973) duration studies in rats, and in two-generation reproductive studies in rats (Bernard et al., 2002). Many studies have shown that PCP causes liver necrosis in experimental animals, but no systematic studies to elucidate whether necrosis is followed by DNA resynthesis have been conducted.

Chronic inflammation is another stimulus that can lead to cell regeneration. Several studies have shown chronic inflammation to occur in liver, olfactory epithelium, and skin of PCP-exposed laboratory animals, but, again, no studies were identified that demonstrate for PCP that this event was a precursor of cell proliferation. However, Umemura et al. (1996) demonstrated that 2–4 weeks of PCP administration to mice resulted in increased DNA content and BrdU labeling of liver cells. Dose- and time-dependent elevation of 8-OH-dG combined with an increase of DNA in the liver, indicating hyperproliferation, suggests that oxidative DNA damage following PCP administration may lead to cellular proliferation that, if sustained, could lead to tumorigenesis in the livers of mice.

Sai et al. (2001, 2000, 1998) demonstrated that aPCP, via decreased levels of the p53 tumor suppressor, inhibited GJIC. Gap junctions form between cells with the help of specialized proteins, CXs. These junctions allow many molecules to pass from one cell to another, enabling one cell to supply the other with metabolites required for survival, or, in the case of apoptosis, to transfer what has been called the death signal, triggering programmed death in cells that are attacked or damaged by certain toxicants. If a chemical prevents gap junctions from forming,

programmed cell death may not occur in a transformed cell that will eventually undergo clonal expansion and develop into a tumor. Many tumor promoters, such as the phorbol esters or PB, have been shown to inhibit GJIC, while other substances that inhibit tumor development, such as corticosteroids or retinoids, have been shown to strengthen GJIC. Specifically, Sai et al. (2001) found that PCP inhibited apoptosis and that this coincided with a 60% drop in the cellular level of p53. The 8-OH-dG moiety in DNA can lead to base-pair exchanges that result in p53 gene mutations. PCP- or PCP metabolite-induced DNA damage, inhibition of GJIC, and increased cellular proliferation have all been shown to be reduced by antioxidants. Considering that PCP can reduce glutathione levels, the results reported by Sai et al. (2001, 2000, 1998) provide support for another mechanism by which PCP potentially promotes DNA damage.

A promoting effect of PCP has also been demonstrated in in vivo studies. In a study designed to look at initiation and promotion activity, Umemura et al. (1999) found that PCP exerted a promoting, but not initiating, effect on mouse liver carcinogenesis. Chang et al. (2003) found that PCP or TCHQ applied repeatedly to mouse skin promoted skin tumor development.

Conclusions about the hypothesized MOA. PCP induces tumors in rodents and there is some evidence of carcinogenicity in humans; however, available experimental information does not support the identification of key events in the MOA of PCP carcinogenicity. The potential for PCP to induce oxidative DNA damage is mostly supported by a few animal and in vitro studies. The available evidence suggests that PCP's para- and possibly orthohydroquinone and benzoquinone metabolites are the principal biologically reactive intermediates. These intermediates can form direct DNA adducts; however, because there is weak evidence for PCP-induced direct mutations in traditional tests, the intermediates are likely unstable. The hydroquinone/benzoquinone metabolites undergo redox cycling resulting in the formation of ROS and 8-OH-dG that in turn can result in chromosomal damage. SCEs, CAs, and SSBs have been demonstrated in animals in vivo and in cell culture, but similar evidence in PCP-exposed humans has been less than conclusive. The influence of oxidative stress on the DNA-damaging action by PCP is supported by reduction of these effects with the application of ROS scavengers and other antioxidants (Lin et al., 2001; Jansson and Jansson, 1992).

The available data suggest that PCP enters the cell and interacts with multiple targets, with oxidative stress involved in both metabolism and proliferative signals. Damaged DNA can lead to apoptosis, necrosis, inappropriate replication, CAs, SCEs, gene mutations, and DNA strand breaks. It is possible that tumors could arise from cells that progressed through mitosis with damaged DNA and failed cell cycle arrest.

Indicators of oxidative stress that were observed in studies with PCP have also been identified in human cancers. The presence of 8-OH-dG and ROS (via oxidative phosphorylation, P450 metabolism, redox cycling, etc.) as well as the formation of DNA adducts have been noted in human carcinogenesis (Klaunig et al., 1998). Other mechanisms such as decreased GJIC have been measured in the cancer process and observed in human carcinogenesis (Trosko and Ruch,

1998; Krutovskikh and Yamasaki, 1997). Oxidative stress is believed to play a role in human carcinogenicity (Loft and Møller, 2006; Klaunig and Kamendulis, 2004; Klaunig et al., 1998; Trush and Kensler, 1991), although the mechanisms involved and the extent to which oxidative stress contributes are not fully understood. The available evidence in animals suggests that the metabolites TCHQ and TCBQ, as well as ROS formed in the course of redox cycling of these metabolites, are involved in PCP-induced carcinogenicity in mammalian cells. However, information on the metabolism of PCP to the quinone metabolites is limited and the level of metabolite(s) associated with a dose of PCP cannot be quantified. It is plausible that long-term exposure to PCP may induce gradual accumulation of oxidative DNA damage in the liver by overwhelming the repair potential and this cumulative oxidative DNA damage could cause critical mutations leading to carcinogenesis; however, the key events are unknown. While data are limited and the MOA by which PCP exerts its carcinogenic effect cannot be characterized, the available evidence in both animals and humans suggests that induction of both indirect and direct DNA damage and subsequent carcinogenicity via oxidative stress is possible. The available data indicate that multiple modes of action for carcinogencity are possible, but none have been defined sufficiently (e.g., key events for carcinogenicity, temporal relationships) to inform the human relevance or low-dose extrapolation for the estimate of the carcinogenicity of PCP.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

4.8.1.1. *Evidence in Humans*

There are a number of cases from poison control centers, as outlined in Section 4.1, where children have been exposed to PCP. In the cases involving small children, no serious outcomes were reported, and in the cases with older children, only one case required critical care. However, an incident where newborns in a nursery were accidentally exposed to PCP via their diapers resulted in severe illness with two fatalities. Blood and tissue measurements of PCP in affected or deceased children showed extreme PCP levels; almost 12 mg/100 mL serum in one child who survived, and tissue levels in excess of 3 mg/100 g tissue in one of the fatalities.

Biomonitoring studies have shown higher levels of PCP in children compared with similarly exposed adults, although differences in toxicological response based on these higher levels are unknown. Kutz et al. (1992) reported higher urinary levels of PCP in adolescents compared to adults, using data from the National Health and Nutrition Examination Survey, a representative sample of the United States population. A study on residents of PCP-treated log homes (Cline, 1989) also found higher serum PCP levels in children compared with their parents. The contribution of biological differences and of differences in exposure to this observed age difference is unknown. One other study of 69 participants, ages 6–87 years (mean 54.6 years), in

Saskatchewan, Canada, did not observe any age-related difference in urinary PCP concentrations (Treble and Thompson, 1996).

There are some data from epidemiologic studies suggesting a susceptibility to adverse health effects (birth defects or childhood cancers) from paternal-mediated exposure during the preconception or perinatal periods. A case-control study in Taiwan reported strong associations (adjusted ORs ≥12.0) with childhood leukemia (103 cases) in relation to paternal work as a wood treater in the pre-conception and perinatal periods (Ali et al., 2004), but there was no association (RR = 1.0) between paternal exposure to PCP and the incidence of childhood leukemia (11 cases) in the large sawmill worker cohort study (Demers et al., 2006; Heacock et al., 2000). Another study of the pregnancy outcomes within this sawmill cohort reported associations between paternal exposure (3 months prior to conception and during the pregnancy) and congenital anomalies of the eye (Dimich-Ward et al., 1996).

4.8.1.2. *Evidence of Reproductive/Developmental Toxicity and Teratogenicity in Animals*

Early studies of reproductive or developmental toxicity suggested that PCP is fetotoxic and teratogenic (Williams, 1982), but these findings were attributed to the chlorinated dibenzo-p-dioxin and dibenzofuran contaminants. However, a considerable number of studies exist where laboratory animals or livestock were exposed to both contaminated and pure PCP during pregnancy, indicating that the contaminants are not solely responsible for the observed fetotoxic effects. A one-generation study in rats (Schwetz et al., 1978) produced evidence of fetotoxicity at maternally toxic doses, but also produced evidence of skeletal variations, and of neonatal toxicity when exposure of the offspring was extended through lactation. A two-generation study in rats (Bernard et al., 2002) showed evidence of hepatotoxicity from PCP in the offspring. Fertility was decreased at high doses, some maturational landmarks were delayed in male and female offspring, and there was evidence for interference with testicular development. Increased maternal body temperature and resorptions and decreased fetal weights were observed in rats exposed on various days of pregnancy to aPCP or tPCP (Larsen et al., 1975). Dosing on GDs 9 or 10 induced the highest level of fetotoxicity. No fetal malformations were observed, and the authors attributed the fetal effects to maternal toxicity.

Two studies of the reproductive toxicity of PCP were performed in mink (Beard and Rawlings, 1998; Beard et al., 1997). Sex hormone levels in females of the F0 generation were measured, but no changes were observed. However, short-term exposure to PCP (Beard et al., 1997) reduced reproductive efficiency of the dams at a dose that was 10 times lower than the dose that caused developmental toxicity in rats (Bernard et al., 2002). Reproductive efficiency of mink was not affected with long-term exposure to PCP (Beard and Rawlings, 1998). However, testicular toxicity consisting of interstial cell hyperplasia and testes length was noted in F1 generation male mink, but they were not as severe in the F2 generation (Beard and Rawlings, 1998).

4.8.1.3. *Evidence of Thyroid Hormone Perturbation in Animals*

McConnell et al. (1980) showed that exposure of 10–14-month-old Holstein cattle to PCP for 160 days resulted in significantly lowered levels of the thyroid hormones T_4 and T_3. Beard et al. (1999b) exposed pregnant rams to PCP and found effects on genital development in the male offspring. T_4 levels were temporarily decreased during the postnatal period, but other hormone levels were not affected. The authors suggested that the lowered T_4 levels were to blame for the impaired sexual development of the males. Beard et al. (1999a) conducted a one-generation reproductive study in sheep exposed to PCP. Reproductive function of the ewes (the rams were not exposed) was not affected by PCP, although T_4 levels were significantly reduced. The significant thyroid hormone-lowering effect of both aPCP and tPCP has also been demonstrated in nonpregnant female rats (Jekat et al., 1994). Beard and Rawlings (1998) reported significant decreases in serum T_4 in mink fed 1 mg/kg-day PCP.

Changes in thyroid hormones have been associated with effects (i.e., delayed myelination, neuronal proliferation, and synapse formation) on neurons. Considering that thyroid hormones may play a role in neurodevelopmental processes, the disruption of thyroid homeostasis that has been observed with PCP indicates a potential concern for the critical period of development of the nervous system (CalEPA, 2006). However, the downstream effects associated with PCP and decreased T_4 levels have not been explored.

A study on pregnant women in Germany has correlated gynecological hormonal effects−specifically, lower T_3 levels−with PCP exposure (Gerhard et al., 1999). No conclusive data exist in support of an estrogenic action of PCP that would be of special concern to humans. Findings in various animal species exposed to PCP point in the same direction, but no evidence has been presented in human or animal carcinogenicity evaluations to suggest that PCP-induced low thyroid hormone levels would be associated with thyroid cancers.

4.8.1.4. *Other Considerations*

One interesting aspect emerges from one of the CYP450 isozymes, CYP3A4, which is thought to metabolize PCP in humans (Mehmood et al., 1996). This enzyme is not expressed in humans before birth; instead, humans express a fetal form, CYP3A7, which exists for a limited time after birth. By 1 year, only CYP3A4 can be found (Williams et al., 2002). Considering that the metabolites of PCP may be the active form of the compound, if CYP3A4 is not present to metabolize PCP (this information is unavailable), it is possible that PCP would be less toxic in humans before they begin to express CYP3A4. An evaluation of published drug clearance data indicates that clearance of drugs metabolized by CYP3A4 is 3 times lower in neonates compared with adults, while in children 1–16 years of age, it is about 1.4 times that of adults (Dorne et al., 2005; Dorne, 2004). If the metabolites are responsible for the toxic effects, the latter age group would have an increased risk for PCP-induced toxicity.

EPA's (2005b) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* refers to stop-exposure studies as possible sources of information concerning childhood susceptibility. The NTP (1999) rat bioassay included one dosing regimen where male and female rats were exposed to the same cumulative dose, either 60 mg/kg-day for 1 year or 30 mg/kg-day for 2 years (all animals were sacrificed at 105 weeks). In contrast to the mouse bioassay (NTP, 1989), where the animals were first dosed at 9 weeks of age, the rats were first dosed at 6 weeks, an age that is considered juvenile. In this study, an elevated incidence of tumors, mesotheliomas, and nasal squamous cell carcinomas was observed exclusively in males subjected to the stop-exposure regimen. The findings of the stop-exposure study (NTP, 1999) suggest that young rats may be more susceptible to the toxicity of PCP delivered at a high-dose rate.

Data suggest that PCP exposure may result in oxidative DNA damage leading to the formation of cancers. Few data are available that describe young animals or children's ability to repair oxidative stress-induced DNA damage compared with adults. Thus, young animals or childrens may be more susceptible to the carcinogenicity of PCP. However, a mitigating factor is that cell replication and mitotic indices are higher in young organisms than in adults; however, because these processes tend to promote the propagation of cells with DNA damage or mutations, it may be assumed that suitable repair mechanisms are in place to prevent that from happening.

4.8.1.5. *Conclusions Concerning Childhood Susceptibility*

Evidence in laboratory animals exists to support some reproductive or developmental toxicity of PCP in laboratory animals. PCP is a weak teratogen, if at all. Many of the effects reported in fetuses may be linked to maternal toxicity and/or the uncoupling of oxidative phosphorylation by PCP. However, the thyroid hormone-lowering effect of PCP seen in animals, and corroborated in one study in human females, is a matter of concern, as low thyroid

levels during pregnancy are known to adversely affect child development (cretinism as the extreme outcome).

It is unknown if the thyroid hormone-lowering and porphyrogenic effects of PCP have any potential impact on cancer development in children. One of the possible MOAs for PCP-induced cancer, oxidative DNA damage, may have a more profound impact in children compared with adults considering the greater activity (1.4 times higher) of the CYP3A4 pathway in humans 1-16 years of age compared with adults. In humans, however, CYP3A4 activity can vary at least 20-fold (Kadlubar et al., 2003; von Ahsen et al., 2001). In the absence of any knowledge concerning the metabolism of PCP at early life stages, pre- and postnatal development of DNA repair systems, control of cell proliferation, and plasticity of the immune system in humans, it is not known whether children are at an increased risk of PCP-induced cancer.

4.8.2. Possible Gender Differences

There is some indication that PCP is a testicular toxicant in rats (NTP, 1999) and mink (Beard and Rawlings, 1998). Few published studies have directly compared the effects of PCP exposure in males and females. Most studies in which PCP was administered to both sexes of a species did not provide substantial or consistent evidence for a difference in gender susceptibility toward the toxicity of PCP. However, both of the NTP bioassays in mice (NTP, 1989) and rats (NTP, 1999) found that males were more susceptible to PCP than females for many of the examined endpoints.

The Hazardous Substances Data Bank (HSDB), an online database of the National Library of Medicine (NLM), lists a 20% higher LD_{50} for female rats (175 mg/kg) as compared with male rats (146 mg/kg) (NLM, 2006). Braun et al. (1977) reported that the toxicokinetics of PCP differed between male and female rats, with elimination rate constants in females being 20–30% higher than in males. This finding could explain the slightly lower toxicity of PCP in female rats.

The NTP stop-exposure study (NTP, 1999) found some sex-related differences in tumor susceptibility. Increased incidences of nasal squamous cell carcinomas and mesotheliomas were observed in male but not female rats. Given that females were less susceptible to PCP toxicity than males, this may indicate that a sufficiently high dose was not achieved in females. The NTP mouse feed study (NTP, 1989) produced similar types of liver cancer in both genders, although only females had elevated incidences of hemangiomas or hemangiosarcomas in the liver and spleen. MOA information to explain gender differences is not available.

Two epidemiologic studies conducted on PCP-exposed women in Germany (Gerhard et al., 1999; Karmaus and Wolf, 1995) suggest that PCP may affect pregnancy and pregnancy outcome. Significantly lowered FSH and T_3 levels in pregnant, PCP-exposed women compared with levels in unexposed pregnant women were reported in one study (Gerhard et al., 1999).

Both studies evaluated women exposed to tPCP used as a wood preservative that contained other toxic agents as contaminants. Because men were not examined in these studies, it cannot be determined whether the observed hormone disturbances are specific to women. Dimich-Ward et al. (1996) present epidemiologic evidence for an uncommon paternally transmitted developmental toxicity in PCP-exposed male workers, suggesting that PCP could be a male reproductive toxicant.

4.8.3. Other Susceptible Populations

No published experimental animal or human epidemiological studies are available to evaluate the effects of PCP in a geriatric population or in individuals with a compromised health status, such as asthmatics, or those with respiratory impairments. A German language retrospective study (Lohmann et al., 1996; English abstract only) examined possible correlations among exposures to certain environmental contaminants, neurotoxicity, and multiple chemical sensitivity (MCS). In almost two-thirds of the cases, exposure to PCP or lindane was associated with symptoms of neurotoxicity and MCS. The authors emphasized that their study was not based on a full-fledged epidemiologic evaluation and was therefore purely descriptive. However, it may be suggested that the condition of MCS heightens the sensitivity to neurotoxic effects in humans exposed to wood preservatives.

Many animal studies provide evidence that it is not the parent compound itself but hydroquinone and benzoquinone metabolites of PCP that are the biologically reactive intermediates. This implies that metabolism is required for toxicity to occur. Mehmood et al. (1996), using yeast cells expressing human CYP450 isozymes, identified CYP3A4 as one isozyme that can metabolize PCP. Metabolism studies in animals using inducers for specific CYP450 isozymes, however, indicated that more than one isozyme is responsible for PCP metabolism (Tsai et al., 2001; Van Ommen et al., 1986a, b). In humans, CYP3A4 activity varies at least 20-fold and displays gene polymorphism, with numerous known variants (He et al., 2005; Kadlubar et al., 2003; Hsieh et al., 2001; von Ahsen et al., 2001). Some of the variants whose catalytic activities have been investigated differ by factors of about two (He et al., 2005; Amirimani et al., 2000). However, there are also a number of mutant alleles with no catalytic activity at all (Hsieh et al., 2001). Because these alleles occur very rarely, it may be concluded that, for CYP3A4 at least, gene polymorphism does not contribute greatly toward a specific susceptibility of humans to PCP-induced toxicity. Other enzymes involved in the metabolism of PCP, such as sulfotransferases or glucuronidases, have not been characterized in detail to warrant an extensive examination of possible gene polymorphisms.

5. DOSE-RESPONSE ASSESSMENT

5.1. ORAL REFERENCE DOSE (RfD)

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

In the absence of human studies on the noncancer effects of PCP, toxicity studies in experimental animals were considered as the basis for the derivation of the oral RfD for PCP. The numerous acute, subchronic, and chronic studies characterizing the systemic toxicity of oral exposure to PCP have been performed in rats, mice, dogs, pigs, rabbits, cattle, mink, and sheep. The primary target for PCP toxicity with both analytical- and commercial-grade formulations was consistently identified by the available animal studies as the liver. Hepatotoxicity has been observed in various animal species after both short- and longer-term exposure to PCP. Other effects have been reported, including reproductive and developmental toxicity, kidney toxicity, neurotoxicity, immunotoxicity, and endocrine effects at doses equal to or greater than those doses eliciting hepatotoxicity.

Many studies in the PCP database were considered to be of limited suitability for derivation of the oral RfD based on incomplete examination of the animals; failure to report grade, purity, and effects of PCP; and/or the use of only one experimental dose of PCP. The remaining studies consist of five chronic studies: three in rats (NTP, 1999; Kimbrough and Linder, 1978; Schwetz et al., 1978), one in mice (NTP, 1989), and one in dogs (Mecler, 1996). Additionally, there are five developmental and reproductive studies in rats (Bernard et al., 2002; Bernard and Hoberman, 2001; Welsh et al., 1987; Schwetz et al., 1978, 1974a).

The Mecler (1996) study examined the toxic effects of tPCP in dogs fed 1.5, 3.5, or 6.5 mg/kg-day tPCP. Decreased absolute body weight (9%) in females was noted at 1.5 mg/kg-day, and mean body weight and body weight gain continued to decline in both male (decreased 4, 6, and 18% at 1.5, 3.5, and 6.5 mg/kg-day, respectively) and female dogs (decreased 13 and 20% at 3.5 and 6.5 mg/kg-day, respectively) as the dose increased. Hepatotoxic effects were noted at 1.5 mg/kg-day with increased incidence of liver pigmentation (in 100% of males and females) consistent with LF, cytoplasmic vacuolation (25% of males, 75% of females), chronic inflammation (100% of males, 50% of females), and severely dark, discolored livers (25% of males, 75% of females) accompanied by significantly increased serum ALP activity (twofold increase over controls for both sexes), and significantly increased relative liver weight in males (14%) and females (37%), and absolute liver weight in females (24%). Absolute liver weight was increased in males (10%) but was not considered statistically significantly greater than controls. As the dose of tPCP increased, the effects observed in the animals of the 1.5 mg/kg-day dose group increased in incidence and severity. Additional effects observed at the 3.5 and 6.5 mg/kg-day doses include increases in serum activity of ALP (2.3- and 4.9-fold in males and 2.6- and 6.8-fold in females at 3.5 and 6.5 mg/kg-day, respectively), ALT (2.8- and 3.9-fold in males and 3.1- and 8.8-fold in females at 3.5 and 6.5 mg/kg-day, respectively), and AST (1.2-

and 1.25-fold in males and 1.1- and 1.7-fold in females, respectively), and minimum hepatocellular necrosis (25% of males, 50% of females). Additionally, foci of hepatocellular hypertrophy, hyperplasia consistent with cirrhosis, fibrosis and decreased hematological parameters (including RBC count, hemoglobin, and hematocrit) were noted in the treated animals. The two animals that were sacrificed in extremis due to morbidity following exposure to tPCP at 6.5 mg/kg-day were characterized as moribund from hepatic insufficiency (Mecler, 1996). The LOAEL was 1.5 mg/kg-day (lowest dose tested), based on dose-related increases in incidence of hepatocellular pigmentation, cytoplasmic vacuolation, chronic inflammation, and severely discolored livers accompanied by statistically significantly increased relative liver weights and serum enzymes, and increased absolute liver weights (significant in females). A NOAEL was not established.

Kimbrough and Linder (1978) fed tPCP and aPCP to male and female rats for 8 months in the diet. A decrease in final body weight (15–16% in tPCP-treated animals; 5 and 10% in aPCP females and males, respectively) and dose-related increases in incidence of liver lesions, including hepatocyte hypertrophy, vacuolation, pleomorphism, periportal fibrosis, abundant brown pigment in macrophages and Kupffer cells, bile duct proliferation, adenofibrosis, and cytoplasmic hyaline inclusions, were observed in rats exposed to doses starting at 2 mg/kg-day for tPCP and at 44 or 48 mg/kg-day (males and females, respectively) for aPCP; however, no incidence data for these effects were reported. Effects were more severe in rats treated with tPCP. The LOAELs, based on hepatotoxicity, were 2 mg/kg-day for males and females exposed to tPCP and 44 and 48 mg/kg-day for males and females, respectively, exposed to aPCP. The NOAEL could not be determined for tPCP. The NOAELs were 9 and 10 mg/kg-day for male and females, respectively, exposed to aPCP.

NTP (1999) reported significantly increased cystic degeneration of hepatocytes in 56 and 78% of males following administration of 20 and 30 mg/kg-day aPCP and eosinophilic focus in 18% of males at 30 mg/kg-day aPCP. Increased centrilobular hepatocyte hypertrophy was noted in 60% of males and females and cytoplasmic hepatocyte vacuolization was observed in 80% of males examined in an interim evaluation after 7 months of administration of 60 mg/kg-day. Increases in serum activity of ALT (1.5-fold for males, 1.1-fold for females), ALP (1.2-fold for males, 1.1-fold for females), and sorbitol dehydrogenase (1.9-fold for males, 1.4-fold for females) were measured in rats administered 60 mg/kg-day aPCP for 7 months. After 2 years (only 1 year of exposure), male rats exhibited increased incidences of liver lesions including: basophilic focus (62%), chronic inflammation (68%), cytoplasmic vacuolization (26%), and cystic degeneration of hepatocytes (56%) at 60 mg/kg-day aPCP. In females, clear cell focus (32%) and cytoplasmic vacuolization (18%) were slightly increased after 1 year of treatment with 60 mg/kg-day followed by 1 year of nontreatment. EPA determined that the LOAEL was 20 mg/kg-day for male rats based on liver toxicity; the NOAEL was 10 mg/kg-day. The LOAEL

was 30 mg/kg-day for female rats based on a biologically significant decrease in body weight; the NOAEL was 20 mg/kg-day.

Rats treated with 1, 3, 10, or 30 mg/kg-day EC-7 (Schwetz et al., 1978) for approximately 2 years exhibited slight increases (~1.7-fold) in serum ALT activity at 30 mg/kg-day. Pigment accumulation in the centrilobular hepatocytes of the liver occurred in 30 and 59% of females given 10 and 30 mg/kg-day. Similarly, 26 and 70% of females receiving 10 and 30 mg/kg-day EC-7 exhibited pigment accumulation in the epithelial cells of the proximal convoluted tubules in the kidney. The study authors reported that the LOAEL was 30 mg/kg-day for males and 10 mg/kg-day for females, based on pigment accumulation in the liver and kidney. The NOAEL was 10 mg/kg-day for males and 3 mg/kg-day for females.

NTP (1989) reported an increased incidence of liver lesions, including clear cell focus (23 and 40%), acute diffuse necrosis (87 and 98%), diffuse cytomegaly (100% for both formulations), diffuse chronic active inflammation (89 and 75%), and multifocal accumulation of brown pigmentation (LF and cellular debris) in Kupffer cells (96 and 83%) in male mice administered 18 mg/kg-day tPCP and EC-7, respectively. Incidence of lesions generally increased with increasing dose. Female mice exhibited clear cell focus (6 and 4%), acute diffuse necrosis (90 and 42%), diffuse cytomegaly (98 and 74%), diffuse chronic active inflammation (69 and 8%), and multifocal accumulation of brown pigmentation (76 and 65%) at doses of 17 mg/kg-day for tPCP and EC-7, respectively. Similar to male mice, the incidence of hepatic lesions in females increased with increasing dose. EPA determined that the LOAELs were 18 mg/kg-day for males and 17 mg/kg-day for females for both tPCP and EC-7. NOAELs could not be established for either tPCP or EC-7 because effects in the liver occurred at the lowest doses tested in male and female mice.

Results of studies that examined the effects of PCP on the liver indicate that rats, mice, and rabbits (NTP, 1999, 1989; Kimbrough and Linder, 1978; Schwetz et al., 1978) are less sensitive to the hepatotoxicity of PCP than the beagle dog (Mecler, 1996). Hepatotoxic effects were observed in rodent and rabbit studies at doses that exceeded those that caused effects in dogs. Specifically, Mecler (1996) reported that a 1-year exposure to tPCP at a concentration of 1.5 mg/kg-day induced hepatotoxicity characterized by increases in hepatic lesions (including liver pigmentation, cytoplasmic vacuolation, chronic inflammation, and the appearance of dark, discolored livers) accompanied by increases in absolute and relative liver weight and serum activity of ALT and ALP in male and female dogs.

Reproductive evaluation of PCP (EC-7) toxicity revealed treatment-related effects in rats at doses of 30 mg/kg-day (Bernard et al., 2002; Schwetz et al., 1978). Decreased parental (8 and 10% in males and females, respectively) and fetal body weight (14–27%), reduced number of pups born alive (6%), pup survival (79%), and increased fetal skeletal variations (quantitative data not reported) were observed at 30 mg/kg-day in a study of rats exposed to 0, 3, or 30 mg/kg-day of PCP (Schwetz et al., 1978). Bernard et al. (2002) reported reductions of 5.3 and 15% for

body weight in 30 and 60 mg/kg-day tPCP treated parental males, respectively. Parental female body weights were reduced 8.3% in the 60 mg/kg-day tPCP dose group. Body weights of the F1 generation rats were reduced 10 and 30% in males and 6 and 23% in females at 30 and 60 mg/kg-day, respectively. Increased liver weight, enlarged liver, centrilobular hypertrophy/vacuolation (100% of males and females), multifocal inflammation (20 and 57% of males; 62 and 63% of females), single-cell necrosis (13 and 70% of males; 38 and 80% of females), and pigmentation (LF; 13 and 37% of males; 45 and 87% of females) were observed in parental rats treated with 30 and 60 mg/kg-day, respectively. Centrilobular hypertrophy (76% of males; 43% of females), pigmentation (10% of females), and multifocal inflammation (7% of males; 13% of females) were observed at the 10 mg/kg-day dose of tPCP. Preputial separation was delayed (~2 days) and spermatid count decreased (10%) in F1 males in the 30 mg/kg-day dose group, while vaginal patency was delayed 1 day in females of the 10 mg/kg-day dose group. Reproductive effects associated with the F1 generation included decreases in live litter size (22%) and viability index (94.4% versus 98.8% in controls) at 60 mg/kg-day; a dose that exceeded that of parental toxicity. The F2 generation presented similar reproductive effects at 60 mg/kg-day (Bernard et al., 2002).

Bernard and Hoberman (2001) reported reductions in maternal (15%) and fetal body weight (79% of controls) and litter size (86% of controls) and increased resorptions (83% of dams versus 41% of controls), and visceral (27%) and skeletal malformations/variations (96%) in rats developmentally exposed to 80 mg/kg-day of tPCP. Decreased maternal body weight gain (22 and 74% for tPCP and aPCP, respectively) and fetal effects, including decreased body weight and crown-rump length (13 and 22% for tPCP and aPCP, respectively), and increased resorptions (27% of fetuses and 95% of litters for tPCP; 97% of fetuses and 100% of litters for aPCP) were observed in rats administered 30 mg/kg-day (Schwetz et al., 1974a). The incidence of delayed ossification of the skull (threefold increase over controls) was noted at a lower dose (5 mg/kg-day) by Schwetz et al. (1974a). Similar to the other developmental studies, Welsh et al. (1987) reported a decrease in maternal body weight gain (76% of control) and the number of viable fetuses (99% decrease) at 43 mg/kg-day of aPCP. Rats exposed to 13 mg/kg-day PCP exhibited an increase in percentage of females with one or more (87.5% of treated versus 67.74% of controls) or two or more resorptions (81.25% of treated versus 41.94% of controls), and fetuses showed an increase in incidence of misshapen centra (36%), and at least two skeletal variations (2.4-fold increase over controls) (Welsh et al., 1987). A developmental study in rabbits showed slight, but significant, decreases in maternal body weight gain of 12 and 29% at 15 and 30 mg/kg-day tPCP, respectively (Bernard et al., 2001).

Reproductive and developmental effects in rodents and rabbits as well as additional effects (kidney, immunological, and neurological; see Section 4.6.1 for more detailed discussion) occurred at doses of PCP that exceeded the doses that elicited hepatotoxicity in dogs (as reported by Mecler, 1996). Therefore, the chronic study by Mecler (1996) in male and female beagle

dogs was selected as the principal study for RfD derivation as it identified effects (hepatotoxicity) at the lowest dose of any of the available studies. The EPA established a LOAEL of 1.5 mg/kg-day based on hepatotoxicity in dogs (Mecler, 1996) characterized by dose-related increases in incidence and severity of pigmentation, cytoplasmic vacuolation, chronic inflammation, and severely discolored livers accompanied by increased relative liver weight and serum enzymes, and increased absolute liver weight (statistically significant in females).

5.1.2. Methods of Analysis—NOAEL/LOAEL Approach

Hepatotoxicity of PCP was evident in the histopathological results of tPCP administration in dogs of the Mecler (1996) study. The observed hepatotoxicity was present in many of the treated dogs (both male and female) at the lowest dose tested, 1.5 mg/kg-day. These effects were minimally present, if at all, in the control animals. For the 3.5 and 6.5 mg/kg-day doses, the hepatotoxicity was present in all animals that survived and the severity of the effects increased with dose. A NOAEL/LOAEL approach is used to derive the RfD for PCP based on the LOAEL of 1.5 mg/kg-day for hepatotoxicity identified by Mecler (1996) in dogs.

In general, the benchmark dose (BMD) approach is preferred over the NOAEL/LOAEL approach for identifying a point of departure (POD). In this particular case, however, the incidence of two of the key liver effects (i.e., hepatocellular pigmentation in males and females and chronic inflammation in males) increased from 0% in the controls to 100% in the low-dose group, and then remained at 100% in both the mid- and high-dose groups. Because of the 100% response at all doses tested, these data are not amenable to BMD modeling, as none of the dose-response models in BMDS can adequately accommodate this steep increase. Thus, the NOAEL/LOAEL approach was employed to identify the POD.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The derivation of the RfD for liver effects from the 1-year toxicity study in beagle dogs (Mecler, 1996) is calculated from the LOAEL by application of a composite UF as follows:

$$RfD = LOAEL \div UF$$

$$RfD = 1.5 \div 300 = 0.005 \text{ mg/kg} = 5 \times 10^{-3} \text{ mg/kg-day}$$

The composite UF of 300 consists of individual UFs of 10 for intraspecies variation, 10 for interspecies variation, and 3 for the use of a LOAEL instead of a NOAEL. The UFs were applied to the POD as described below:

- A default intraspecies uncertainty factor (UF_H) of 10 was applied to account for variability in susceptibility among members of the human population in the absence of quantitative information on the variability of human response to PCP. Current

information is unavailable to assess human-to-human variability in PCP toxicokinetics and toxicodynamics; therefore, to account for these uncertainties, a factor of 10 was applied for individual variability.

- A default interspecies uncertainty factor (UF_A) of 10 was applied to account for the potential pharmacokinetic and pharmacodynamic differences between dogs and humans. Although toxicokinetic data are available in some animals, a description of toxicokinetics in either dogs or humans is limited or not available. In the absence of data to quantify specific interspecies differences, a factor of 10 was applied.

- An uncertainty factor (UF_L) of 3 was applied to account for the extrapolation from a LOAEL to a NOAEL. The 1.5 mg/kg-day dose level was selected as the LOAEL based on histopathological changes in the liver, consisting of increased incidence of pigmentation in both males and females; minimal chronic inflammation in males; and increased relative liver weights in males and absolute and relative liver weight in females. These effects were accompanied by small changes (less than twofold) in serum enzymes (ALT in males and ALP in males and females), indicating an effect of minimal toxicological significance. Therefore, a factor 3 was applied to account for the use of a LOAEL that is characterized by effects that can be considered mild.

- An UF of 1 was applied to extrapolate from a subchronic to a chronic (UF_S) exposure duration because the RfD was derived from a study using a chronic exposure protocol.

- An UF of 1 was applied to account for database deficiencies (UF_D). The database for PCP contains human studies; chronic studies in rats, mice, and dogs; subchronic studies in various animal species; neurological, reproductive, endocrine, and developmental and reproductive toxicity studies; and a two-generation reproductive toxicity study.

5.1.4. RfD Comparison Information

The predominant noncancer effect of subchronic and chronic oral exposure to PCP is hepatic toxicity. Figure 5-1 provides a graphical display of dose-response information from six studies that reported liver toxicity in experimental animals following chronic oral exposure to PCP, focusing on candidate PODs that could be considered in deriving the oral RfD. As discussed in Sections 5.1.1 and 5.1.2, among those studies that demonstrated liver toxicity, the study by Mecler (1996) provided the most sensitive data set for deriving the RfD. Potential reference values that might be derived from each of the other studies are also presented.

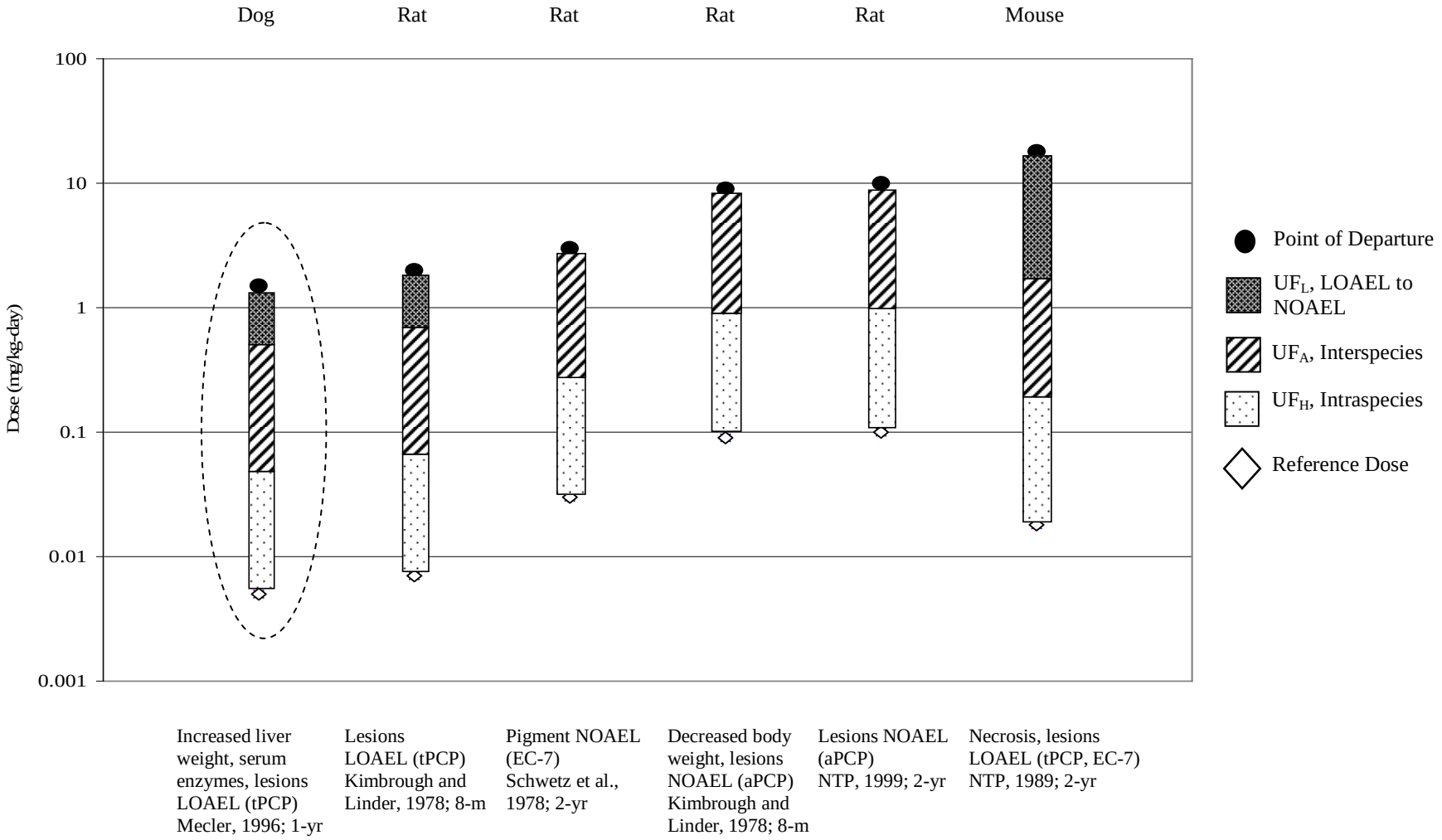

Figure 5-1. Array of candidate PODs with applied uncertainty factors and reference values for a subset of hepatotoxic effects of studies in Table 5-1.

Table 5-1. Candidate PODs for hepatotoxicity with applied UF and potential reference values

Endpoint	Candidate POD (mg/kg-day)	UFs				Potential reference value (mg/kg-day)	Reference
		Composite UF	UF_L	UF_A	UF_H		
Increased liver weight and serum enzymes; hepatocellular lesions; LOAEL Dog, 1 yr	1.5	300	3	10	10	0.005	Mecler, 1996 (tPCP)
Hepatocellular lesions; LOAEL Rat, 8 m	2	300	3	10	10	0.007	Kimbrough and Linder, 1978 (tPCP)
Pigment; NOAEL Rat, 2 yr	3	100	1	10	10	0.03	Schwetz et al., 1978 (EC-7)
Decreased body weight, hepatocellular lesions; NOAEL Rat, 8 m	9 (M) 10 (F)	100	1	10	10	0.09 (M) 0.1 (F)	Kimbrough and Linder, 1978 (aPCP)
Lesions; NOAEL Rat, 2 yr	10	100	1	10	10	0.1	NTP, 1999 (aPCP)
Necrosis, hepatocellular lesions; LOAEL Mouse, 2 yr	18	1,000	10	10	10	0.018	NTP, 1989 (tPCP, EC-7)

Reproductive and developmental studies in experimental animals have found that PCP can produce prenatal loss, skeletal and soft-tissue variations, delays in puberty, and decreased fetal weight; these doses also produced toxic effects in the dams. These studies show that the developing embryo and fetus may be a target of PCP toxicity; however, study results indicate that PCP is more likely to be embryo- and fetotoxic rather than teratogenic. A graphical display of dose-response information from two reproductive and four developmental studies is provided in Figure 5-2. For the reasons discussed above and in Section 5.1.1, liver effects in the dog observed in the study by Mecler (1996) are considered the most sensitive effects to serve as the basis for the derivation of the RfD for PCP. The potential reference value associated with delayed ossification of the skull in fetuses of rats administered 5 mg/kg-day aPCP from GD 6 to 15 (Schwetz et al., 1974a) is identical to the RfD based on hepatotoxicity in dogs administered 1.5 mg/kg-day tPCP (Mecler, 1996). The POD for hepatotoxicity is the same as or lower than that for reproductive and developmental toxicity, and the resulting RfD should protect against reproductive and developmental effects of PCP.

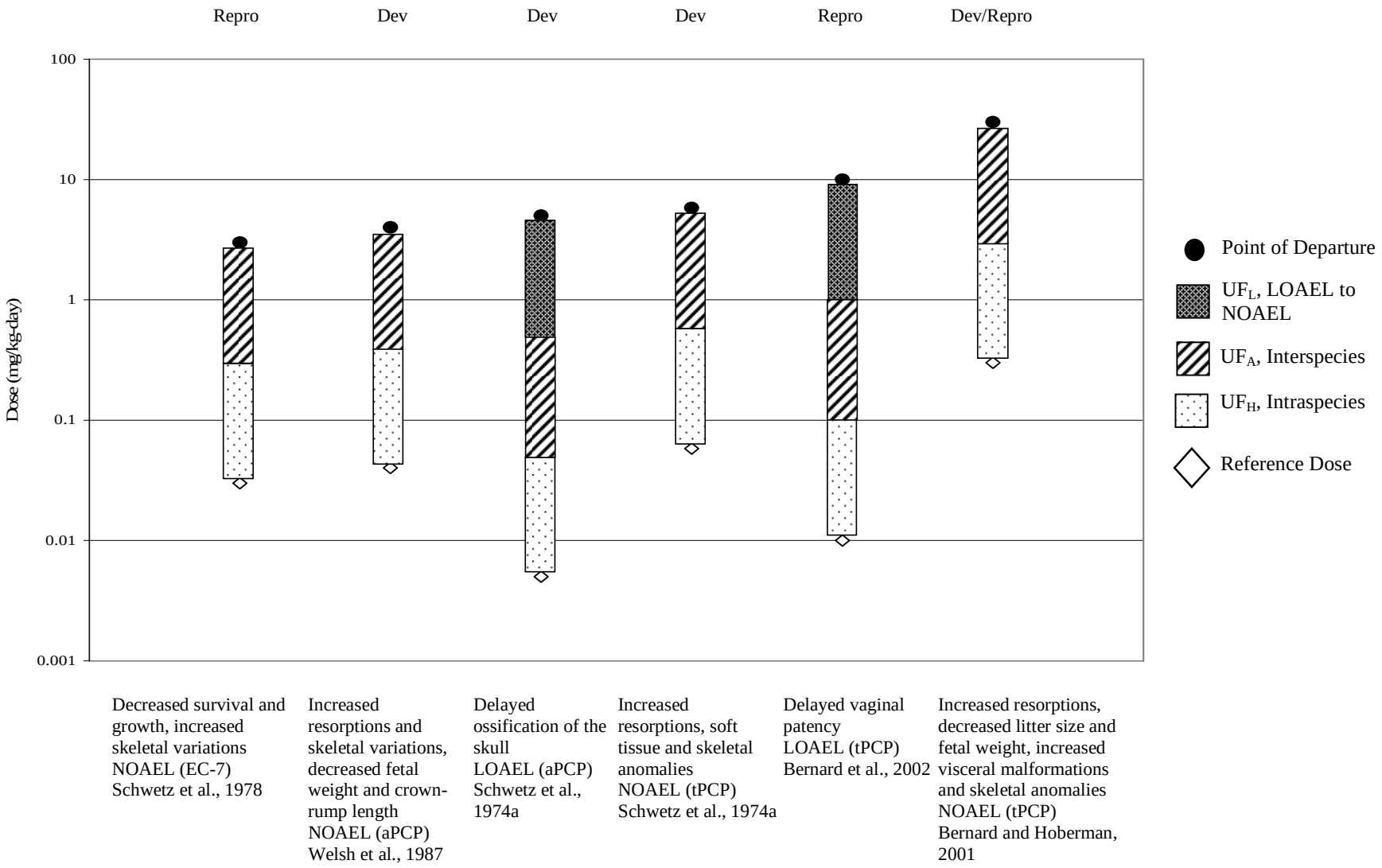

Figure 5-2. Array of candidate PODs with applied uncertainty factors and reference values for a subset of reproductive and developmental effects of studies in Table 5-2.

Table 5-2. Candidate PODs for reproductive and developmental toxicity in rats with applied UF, and potential reference values

Endpoint	Candidate POD (mg/kg-day)	Uncertainty factors (UFs)				Potential reference values (mg/kg-day)	Reference
		Composite UF	UF_L	UF_A	UF_H		
Decreased survival and growth, increased skeletal variations; NOAEL	3	100	1	10	10	0.03	Schwetz et al., 1978 (EC-7)
Increased resorptions and skeletal variations, decreased fetal wt & crown-rump length; NOAEL	4	100	1	10	10	0.04	Welsh et al., 1987 (aPCP)
Delayed ossification of the skull; LOAEL	5	1,000	10	10	10	0.005	Schwetz et al., 1974a (aPCP)
Increased resorptions, soft tissue and skeletal anomalies; NOAEL	5.8	100	1	10	10	0.06	Schwetz et al., 1974a (tPCP)
Delayed vaginal patency; LOAEL	10	1,000	10	10	10	0.01	Bernard et al., 2002 (tPCP)
Increased resorptions, decreased litter size and fetal weight, increased visceral malformations, and skeletal anomalies; NOAEL	30	100	1	10	10	0.3	Bernard and Hoberman, 2001 (tPCP)

5.1.5. Previous RfD Assessment

The previous RfD, posted to the IRIS database in January 1987, was based on a chronic oral rat study by Schwetz et al. (1978). Investigators administered 0, 3, 10, or 30 mg/kg-day PCP in feed ad libitum to 25 rats/sex/dose for 22 (males) or 24 months (females). Derivation of the RfD of 3×10^{-2} mg/kg-day was based on a NOAEL of 3 mg/kg-day for liver and kidney pathology, evidenced by pigmentation of the liver and kidneys in female rats at 10 mg/kg-day (LOAEL). A composite UF of 100 (UF_H of 10 for intraspecies variability and a UF_A of 10 for interspecies variability) was applied to the NOAEL.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

Adequate data are not available to derive an inhalation RfC. No chronic or subchronic animal studies for inhalation exposure are available. The previous IRIS assessment did not derive an RfC.

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE AND INHALATION REFERENCE CONCENTRATION

Uncertainties associated with the RfD in the assessment for PCP are identified in the following discussion. As presented earlier in Section 5.1, UFs were applied to the POD, a LOAEL, for deriving the RfD. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure and for a diverse population of varying susceptibilities. These extrapolations are carried out with default approaches given the limitations of experimental PCP data for the interspecies and intraspecies differences.

A range of animal toxicology data is available for the hazard assessment of PCP, as described in Section 4. Included in these studies are short-term and long-term studies in dogs, rats, and mice and developmental and reproductive toxicity studies in rats, as well as numerous supporting studies. Toxicity associated with oral exposure to PCP is observed as hepatic and reproductive and developmental endpoints. Critical data gaps have been identified in Section 4 and uncertainties associated with data deficiencies are more fully discussed below.

Consideration of the available dose-response data to determine an estimate of oral exposure that is likely to be without an appreciable risk of adverse health effects over a lifetime led to the selection of the 1-year oral study in beagle dogs (Mecler, 1996) as the principal study and hepatotoxicity (characterized by increased incidence and severity of liver pigmentation, cytoplasmic vacuolation, chronic inflammation, and severely discolored livers, significantly increased absolute [females only] and relative liver weights, and increased serum enzyme activity) as the critical effect for deriving the RfD for PCP. The dose-response relationships for oral exposure to PCP and hepatotoxicity in rats and mice are also available for deriving an RfD, but are associated with higher NOAELs/LOAELs that would be protected by the selected critical effect and corresponding POD.

The Mecler (1996) study used a PCP formulation that was 90.0% pure. As discussed in Section 5.4.4, impurities in the formulation could influence the toxicity of the test compound. Whether these impurities would reduce or increase the toxicity relative to aPCP is unknown.

The derived RfD was quantified using a LOAEL for the POD. A POD based on a NOAEL or LOAEL is, in part, a reflection of the particular exposure concentration or dose at which a study was conducted. It lacks characterization of the dose-response curve and for this reason is less informative than a POD obtained from BMD modeling. In this particular case, however, BMD modeling was not utilized for the determination of the POD for hepatotoxicity in Mecler (1996) because the incidence of two of the key liver effects (i.e., hepatocellular pigmentation in males and females and chronic inflammation in males) increased from 0% in the controls to 100% in the low-dose group, and then remained at 100% in both the mid- and high-dose groups. Because none of the dose-response models in BMDS can adequately accommodate this steep increase in response at the lowest dose, it was determined that the critical data set was not amenable to BMD modeling, and the NOAEL/LOAEL approach was used to identify the POD.

The oral reproductive and developmental toxicity studies indicate that the developing embryo and/or fetus may be a target of PCP toxicity. However, observed toxic effects were not teratogenic in nature, but rather embryo- or fetotoxic. Systemic effects were frequently observed in the dams at similar doses. In the two-generation reproductive study, hepatotoxic effects were noted in the dams at doses that elicited delayed vaginal patency in the F1 offspring females. The potential reference value associated with delayed ossification of the skull in fetuses of rats administered 5 mg/kg-day aPCP from GD 6 to 15 (Schwetz et al., 1974a) is identical to the RfD based on hepatotoxicity in dogs administered 1.5 mg/kg-day tPCP (Mecler, 1996). The POD for hepatotoxicity is lower than that for reproductive and developmental toxicity, and the resulting RfD should protect against reproductive and developmental effects of PCP.

A LOAEL was identified based on hepatotoxicity in dogs administered tPCP in Mecler (1996). The hepatotoxicity was observed at all doses, including the lowest dose tested; therefore, a NOAEL was not established. In the absence of an established NOAEL, the LOAEL was used as the POD to derive the RfD. A threefold UF was applied to account for the use of a POD characterized by effects that can be considered mild at the dose established as the LOAEL.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and its magnitude associated with the concentration at the POD in dogs are extrapolated to human response. Pharmacokinetic models are useful for examining species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not available for oral exposure to PCP. Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans, so the 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD.

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. In the absence of PCP-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of the RfD.

5.4. CANCER ASSESSMENT

5.4.1. Choice of Study/Data—with Rationale and Justification

The available epidemiologic studies support an association between PCP exposure and development of specific cancers, i.e., non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, and liver cancer (Section 4.1.1). However, the lack of an exposure estimate that allows for an absolute, rather than a relative, level of exposure, renders these studies unsuitable for deriving cancer risk estimates for PCP via the oral or inhalation routes. The most detailed exposure assessment was in the large cohort study of over 26,000 sawmill workers in British Columbia (Demers et al., 2006). This study used a metric based on a cumulative dermal chlorophenol exposure score, with 1 exposure year defined as 2,000 hours of dermal contact.

Two well-conducted studies provide data for the carcinogenicity of PCP via the oral route in laboratory animals: one study utilizing B6C3F_1 mice (NTP, 1989) and another study in F344 rats (NTP, 1999). Two types of PCP, tPCP and EC-7, were carcinogenic in the mouse. Hepatocellular adenomas/carcinomas and adrenal medullary pheochromocytomas developed in male mice treated with tPCP or EC-7, and hepatocellular adenomas/carcinomas and hemangiosarcomas developed in female mice treated with tPCP or EC-7 and adrenal medullary pheochromocytomas developed in female mice treated with EC-7.

In the mouse study, the carcinogenicity of tPCP, which contains appreciable amounts of chlorinated dibenzo-p-dioxins and dibenzofurans, was compared with the carcinogenicity of EC-7, which contains relatively low levels of the dioxins and furans. Mice were administered tPCP (90.4% purity; 18 or 35 mg/kg-day for males and 17 or 35 mg/kg-day for females) or EC-7 (91.9% purity; 18, 37, or 118 mg/kg-day for males and 17, 34, or 114 mg/kg-day for females) in feed for 2 years. In male mice, the incidence of hepatocellular adenomas and carcinomas combined showed a statistically significantly elevated trend with increasing levels of tPCP and EC-7. In female mice, the incidence of hepatocellular adenomas and carcinomas combined showed a statistically significantly elevated trend with increasing levels of EC-7. The incidence of hepatocellular adenomas and carcinomas combined was statistically significantly elevated only at 114–118 mg/kg-day EC-7 when compared with the control group. The remaining exposures exhibited an increase in hepatocellular adenomas and carcinomas; however, these were not considered statistically significant when compared with control values.

Adrenal gland medullary pheochromocytomas and malignant pheochromocytomas were observed in all dose groups of both tPCP and EC-7 grades of PCP. There was a statistically significant increase in the incidence of combined pheochromocytomas and malignant pheochromocytomas in male mice at all doses of tPCP and all doses of EC-7, except 18 mg/kg-

day. Pheochromocytomas were also observed in female mice administered tPCP and EC-7, although the appearance of tumors in tPCP mice did not exhibit a dose-related increase and the only statistically significant increase in incidence was observed in the 114–118 mg/kg-day EC-7 dose group. A significant positive trend was observed for pheochromocytomas in male mice treated with tPCP and male and female mice treated with EC-7.

Hemangiosarcomas were observed in male mice administered both grades of PCP, although the incidences were slight and not considered statistically significant. Female mice administered tPCP showed an increase in hemangiosarcomas at both doses, but the increase was only significant at the high dose (35 mg/kg-day for tPCP). Increased incidences of combined hemangiomas and hemangiosarcomas were observed in EC-7 females, and the incidence in the high-dose (118 mg/kg-day) group was significantly elevated compared with controls.

The rat bioassay (NTP, 1999) examined the effects of aPCP in male and female F344 rats. There was some evidence of carcinogenicity in the male rat that exhibited a significantly higher incidence of malignant mesothelioma at 60 mg/kg-day (dose used in the 1-year stop-exposure study) compared with that of controls. The incidence exceeded the range of historical controls. The incidence of nasal squamous cell carcinomas was also elevated in 60 mg/kg-day males, and while the incidence did not achieve statistical significance compared with that of concurrent controls, it did exceed the range of historical controls. Nasal squamous cell carcinomas were observed in male rats administered 10 mg/kg-day and were the only neoplastic finding in male rats treated for the full 2 years of the bioassay that occurred with a higher incidence than that of historical controls. However, nasal tumors were not considered treatment-related because the incidence at 20 and 30 mg/kg-day was less than or equal to the control incidence. There were no treatment-related increases in the incidences of tumors in female rats receiving aPCP. This study showed some evidence of carcinogenicity of aPCP in male F344 rats exposed to 60 mg/kg-day aPCP, based on increased incidences of mesothelioma and nasal squamous cell carcinoma in the stop-exposure study.

The mouse study was selected for dose-response assessment based on statistically significant increased incidences of hepatocellular adenomas and carcinomas, adrenal pheochromocytomas and malignant pheochromocytomas, and hemangiomas and hemangiosarcomas (in liver and spleen) at multiple exposure levels in males and females. The study by NTP (1989) was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes of $B6C3F_1$ mice with two grades of PCP (tPCP and EC-7) and with 50 male and 50 female mice per dose group (typical for NTP-type bioassays). The test animals were allocated among two dose levels for tPCP and three dose levels for EC-7 with untreated control groups for each PCP formulation. Animals were observed twice daily and examined weekly (for 12–13 weeks) and then monthly for body weight and monthly for feed consumption. Animals were necropsied and all organs and tissues were examined grossly and microscopically for histopathological lesions. Tumor incidences were elevated with increasing

exposure level at multiple sites in both sexes, including the liver, adrenal gland, and circulatory system.

The male F344 rat tumor incidence data (NTP, 1999), while demonstrating some evidence of carcinogenicity, were not used for deriving low-dose quantitative risk estimates. The responses of increased incidence of mesothelioma and nasal squamous cell carcinoma in male rats were lower than those of the mice (NTP, 1989) at a greater exposure level, suggesting greater sensitivity of the mice. The toxicological database for PCP studies in rodents has shown the mouse model, rather than the rat, to be a more sensitive model of PCP hepatotoxicity. Additionally, the differences in the presence of metabolites, TCpBQ in mice versus TCoBQ in rats and subsequent formation of DNA adducts via TCpBQ that is believed to be associated with the oxidative stress-related toxicity and the proposed MOA, also suggest that the mice are more sensitive than the rats. Although the NTP (1999) bioassay in rats administered aPCP reported mesotheliomas and nasal squamous cell carcinomas, the tumor incidence was statistically significantly elevated only at the high dose (1-year exposure). The lack of a significant dose-response trend in the rat data and the observation of consistently greater sensitivity to PCP in mice, rather than rats, led to the use of the mouse data for the derivation of the slope factor. Consequently, dose-response modeling was not carried out with the rat tumor data.

5.4.2. Dose-Response Data

Oral cancer risk estimates were calculated based on the incidences of hepatocellular and adrenal medullary tumors in male mice, and hepatocellular tumors, adrenal medullary tumors, and hemangiomas/hemangiosarcomas in female mice treated with tPCP or EC-7 (NTP, 1989). Data are not available to indicate whether malignant tumors developed specifically from progression of benign tumors; however, etiologically similar tumor types (i.e., benign and malignant tumors of the same cell type) were combined for these analyses because of the possibility that the benign tumors could progress to the malignant form. Thus, adenomas and carcinomas of the liver were considered together because adenomas develop from the same cell lines and can progress to carcinomas. The adrenal medullary tumors, distinguished as either pheochromocytomas or malignant pheochromocytomas, were also considered together. The classification of malignant pheochromocytoma was assigned if the pheochromocytoma progressed and was observed as obliterating the cortex (outer layer of the adrenal gland) or penetrating the capsule of the adrenal gland. Hemangiosarcomas differed from the hemangiomas in that the hemangiosarcomas consisted of a greater amount of pleomorphic and anaplastic endothelial cells (NTP, 1989); these tumors were also considered together.

The male and female mice were exposed to tPCP and EC-7, two formulations of PCP that are approximately 90% pure. However, the composition of the impurities that have been identified in these two formulations differs both qualitatively and quantitatively. Based on the diversity of contaminants found in the tPCP and EC-7 forms of PCP, these two datasets were

modeled separately. Animals dying before the first appearance of tumors during the first year of exposure in any group of that sex were censored from the group totals when figuring the denominators. This adjustment was made so that the denominators included only those animals at risk for developing tumors. The incidences of tumors in mice treated with tPCP and EC-7 are presented in Table 5-3.

Table 5-3. Incidence of tumors in B6C3F$_1$ mice exposed to tPCP and EC-7 in the diet for 2 years

Tumor type	tPCP, ppm in diet			EC-7, ppm in diet			
	0	100	200	0	100	200	600
	mg/kg-day[a]			mg/kg-day[a]			
Males	**0**	**18**	**35**	**0**	**18**	**37**	**118**
Hepatocellular adenoma/carcinoma	7/32[b] (7/28)[d]	26/47[c] (26/46)	37/48[c] (37/46)	6/35[b] (6/33)	19/48[c] (19/45)	21/48[c] (21/38)	34/49[c] (34/47)
Adrenal benign/malignant pheochromocytoma	0/31[b] (0/26)	10/45[c] (10/41)	23/45[c] (23/44)	1/34[b] (1/32)	4/48 (4/45)	21/48[c] (21/39)	45/49[c] (45/47)
Females	**0**	**17**	**35**	**0**	**17**	**34**	**114**
Hepatocellular adenoma/carcinoma	3/33 (3/31)	9/49 (9/49)	9/50 (9/48)	1/34[b] (1/34)	4/50 (4/49)	6/49 (6/49)	31/48[c] (31/48)
Adrenal benign/malignant pheochromocytoma	2/33 (2/31)	2/48 (2/48)	1/49 (1/47)	0/35[b] (0/35)	2/49 (2/48)	2/46 (2/46)	38/49[c] (38/49)
Hemangioma/hemangiosarcoma	0/35[b] (0/33)	3/50 (3/50)	6/50[c] (6/48)	0/35[b] (0/35)	1/50 (1/49)	3/50 (3/50)	9/49[c] (9/49)

[a]Average daily doses estimated by the researchers.
[b]Statistically significant trend ($p < 0.05$) by Cochran-Armitage test.
[c]Statistically significant difference from controls ($p < 0.05$) by Fisher Exact test.
[d]Censored data used for modeling are shown in parentheses; see text for description of censoring procedure.

Source: NTP (1989).

Following statistical analysis (Fischer Exact and χ^2 tests), the responses in male mice control groups between the tPCP and EC-7 groups were judged to be similar for both hepatocellular and adrenal tumors. Additionally, the responses in female control mice for hepatocellular, adrenal, and circulatory tumors were similar for the tPCP and EC-7 experiments. Therefore, all dose-response analyses were conducted using combined controls.

5.4.3. Dose Adjustments and Extrapolation Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The dose response is assumed to be linear in the lowest dose range when evidence supports a genotoxic MOA because of DNA reactivity, or if another MOA is applicable that is anticipated

to be linear. A nonlinear approach is appropriate when there are sufficient data to ascertain the MOA and conclude that it is nonlinear (e.g., when the carcinogenic action is secondary to another toxic effect that itself has a threshold). The linear approach to low-dose extrapolation is taken for agents where the MOA is uncertain (U.S. EPA, 2005a).

As discussed in Section 4.7.3, the available data indicate that multiple modes of carcinogenic action are possible, but none have been defined sufficiently (e.g., key events for carcinogenicity, temporal relationships) to inform the human relevance or low-dose extrapolation for the carcinogenicity of PCP. Therefore, as recommended in the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005a), "when the weight of evidence evaluation of all available data are insufficient to establish the MOA for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach." Accordingly, for the derivation of a quantitative estimate of cancer risk for ingested PCP, a linear extrapolation was performed to determine the cancer slope factor.

The multistage model has been used by EPA in the vast majority of quantitative cancer assessments because it is thought to reflect the multistage carcinogenic process and it fits a broad array of dose-response patterns. Occasionally the multistage model does not fit the available data, in which case alternatives should be considered. Alternatives include dropping higher exposure groups if, for example, the responses plateau at the higher exposures and the potential POD is in the range covered by the remaining exposure levels. Alternate models may be used if dropping groups is not feasible. Use of this decision scheme has contributed to greater consistency among cancer risk assessments. Consequently, the multistage model was the primary tool considered for fitting the dose-response data and is given by:

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)], \qquad (1)$$

where:

$P(d)$ = lifetime risk (probability) of cancer at dose d

q_i = parameters estimated in fitting the model, i = 1, …, k

The multistage model in U.S. EPA's Benchmark Dose Software (BMDS) (version 1.3.2) (U.S. EPA, 2004) was used for all model fits, and complete results are shown in Appendix D. Adequate fits were obtained for each of the data sets as assessed by the chi-square goodness-of-fit statistic ($p > 0.1$). In one case, adrenal pheochromocytomas for male mice exposed to EC-7, an adequate fit was achieved after dropping the highest exposure group. The BMD modeling results and their 95% lower bounds (BMDLs) derived from each endpoint for the individual data sets are summarized in Table 5-4.

Table 5-4. Summary of BMD modeling for PCP cancer data in male and female B6C3F$_1$ mice

Test material	Sex	Endpoint	Model degree	BMD_{10}[a] (mg/kg-day)	$BMDL_{10}$[b] (mg/kg-day)
tPCP	M	Hepatocellular adenoma/carcinoma	One stage	***3.12***	***2.27***
	M	Adrenal pheochromocytoma/ malignant pheochromocytoma	One stage	6.45	4.47
	F	Hepatocellular adenoma/carcinoma	One stage	21.3	11.7
	F	Hemangioma/hemangiosarcoma	One stage	27.8	16.3
EC-7	M	Hepatocellular adenoma/carcinoma	One stage	11.0	7.59
	M	Adrenal pheochromocytoma/ malignant pheochromocytoma	Two stage	12.6	5.75
	F	Hepatocellular adenoma/carcinoma	Two stage	36.9	16.4
	F	Adrenal pheochromocytoma/ malignant pheochromocytoma	Two stage	45.5	29.6
	F	Hemangioma/hemangiosarcoma	One stage	61.7	37.9

[a]BMDs, calculated using polynomial multistage model of BMDS version 1.3.2, associated with a 10% extra risk.
[b]BMDL = 95% lower confidence limit on the BMD.

Source: NTP (1989).

A $BW^{3/4}$ (body mass raised to the 3/4 power) scaling factor was used to convert the PODs in the mouse study to human equivalent doses (HEDs), in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). This procedure presumes that equal doses in these units (i.e., in $mg/kg^{3/4}$-day), when administered daily over a lifetime, will result in equal lifetime risks of the critical effect across mammalian species (U.S. EPA, 1992). The HED may be calculated as follows (U.S. EPA, 2005a, 1992):

$$HED\ (mg/kg\text{-}day) = dose\ in\ animals\ (mg/kg\text{-}day) \times (BW_a/BW_h)^{0.25}$$

where:

HED = human equivalent dose
Dose = average daily dose in animal study
BW_a = animal body weight (kg)
BW_h = reference human body weight (70 kg)

The time-weighted average body weights in the combined controls were used to represent animal body weights in the above equation (0.037 kg for males and 0.038 kg for females). The cross-species scaling factor of 0.15 was used to calculate the HEDs shown in Table 5-5.

Table 5-5. Summary of $BMDL_{10/HED}$ and cancer slope factors derived from PCP cancer data in male and female $B6C3F_1$ mice (NTP, 1989)

Test Material	Sex	Endpoint	$BMD_{10/HED}$[a] (mg/kg-day)	$BMDL_{10/HED}$[a] (mg/kg-day)	Slope factor[b] $(mg/kg\text{-}day)^{-1}$
tPCP	M	Hepatocellular adenoma/carcinoma	0.475	**0.35**	**2.9×10^{-1}**
	M	Adrenal pheochromocytoma/malignant pheochromocytoma	0.981	0.68	1.5×10^{-1}
	F	Hepatocellular adenoma/carcinoma	3.24	1.79	5.6×10^{-2}
	F	Hemangioma/hemangiosarcoma	4.23	2.48	4.0×10^{-2}
EC-7	M	Hepatocellular adenoma/carcinoma	1.68	1.15	8.7×10^{-2}
	M	Adrenal pheochromocytoma/malignant pheochromocytoma	1.92	0.88	1.1×10^{-1}
	F	Hepatocellular adenoma/carcinoma	5.61	2.50	4.0×10^{-2}
	F	Adrenal pheochromocytoma/malignant pheochromocytoma	6.93	4.51	2.2×10^{-2}
	F	Hemangioma/hemangiosarcoma	9.24	5.76	1.7×10^{-2}

[a]$BMD(L)_{HED} = BMD(L)*BW^{3/4}$ scaling factor.
[b]Cancer slope factor calculated by dividing the risk at the POD by the $BMDL_{HED}$ at the POD ($0.1/BMDL_{10/HED}$).

Source: NTP (1989).

Alternatively, the cross-species scaling factor could have been applied to the individual exposure levels for each dose-response analysis, prior to modeling. When the cross-species factor is the same across groups, because of no appreciable difference in body weights in a data set, it is numerically equivalent to apply the factor after modeling to the BMDs only, as in this assessment.

5.4.4. Oral Slope Factor and Inhalation Unit Risk

A low-dose linear extrapolation approach results in calculation of an oral slope factor that describes the cancer risk per unit dose of the chemical at low doses. The oral slope factors for each data set considered were calculated by dividing the risk at the POD by the corresponding BMDL ($0.1/BMDL_{10/HED}$). The site-specific oral slope factors are summarized in Table 5-5.

The slope factors ranged from 1.7×10^{-2} to 8.7×10^{-2} $(mg/kg\text{-}day)^{-1}$ for EC-7 and from 4×10^{-2} to 2.9×10^{-1} $(mg/kg\text{-}day)^{-1}$ for tPCP. The highest PCP cancer slope factor (2.9×10^{-1} $(mg/kg\text{-}day)^{-1}$) resulted from the analysis of combined incidences for hepatocellular adenomas and carcinomas in tPCP male mice. Considering the multiple tumor types and sites observed in the mice exposed to PCP, the estimation of risk based on only one tumor type/site may underestimate the overall carcinogenic potential of PCP.

EPA's cancer guidelines (U.S. EPA, 2005a, b) identify two ways to approach this issue—analyzing the incidences of tumor-bearing animals, or combining the potencies associated with significantly elevated tumors at each site. The NRC (1994) concluded that an approach based on counts of animals with one or more tumors would tend to underestimate overall risk when tumor types occur independently, and that an approach based on combining the risk estimates from each separate tumor type should be used. The NRC (1994) recommended an approach based on simulations. Therefore, a bootstrap analysis (Efron and Tibshirani, 1993) was used to derive the distribution of the BMD for the combined risk of liver, adrenal gland, and circulatory system tumors observed in male and female mice with oral exposure to PCP. A simulated incidence level was generated for each exposure group using a binomial distribution with probability of success estimated by a Bayesian estimate of probability. Each simulated data set was modeled using the multistage model in the same manner as was done for the individual risks associated with the liver, adrenal gland, and circulatory system tumors. The 5^{th} percentile from the distribution of combined BMDs was used to estimate the BMDL corresponding to an extra risk of 1% for any of the three tumor sites. This analysis is described in greater detail in Appendix E (see Table E-1).

The results of combining risks across sites within datasets are shown in Table 5-6. The highest combined risk observed, similar to the individual cancer risk estimates, was in tPCP-exposed male mice. The male mice were consistently more sensitive than female mice to PCP tumor-induction. The 95% upper confidence limit (UCL) on the combined risk for male mice that developed liver and/or adrenal gland tumors was 4.0×10^{-1} $(mg/kg\text{-}day)^{-1}$, which is about 38% higher than the 2.9×10^{-1} $(mg/kg\text{-}day)^{-1}$ cancer slope factor estimated from liver tumors only in tPCP-exposed male mice. The risk estimates for the tPCP-exposed males and females tend to be higher than those for the EC-7-exposed animals, by approximately twofold for the central tendency estimates and for the upper bound estimates. These differences suggest a slightly greater potency for the technical grade. Several issues bear consideration before recommending a slope factor for oral exposure only to PCP.

Table 5-6. Human-equivalent combined risk estimates for liver, adrenal, and circulatory tumors in B6C3F$_1$ mice

Sex	Endpoints	Human-equivalent combined risk (mg/kg-day)[a]	
		Central tendency	Upper bound
tPCP			
Male	Hepatocellular adenoma/carcinoma or adrenal pheochromocytoma/malignant pheochromocytoma	2.9×10^{-1}	4.0×10^{-1}
Female	Hepatocellular adenoma/carcinoma, adrenal pheochromocytoma/malignant pheochromocytoma, or hemangioma/ hemangiosarcoma	5.2×10^{-2}	8.3×10^{-2}
EC-7			
Male	Hepatocellular adenoma/carcinoma or adrenal pheochromocytoma/malignant pheochromocytoma	1.1×10^{-1}	1.7×10^{-1}
Female	Hepatocellular adenoma/carcinoma, adrenal pheochromocytoma/malignant pheochromocytoma, or hemangioma/hemangiosarcoma	2.8×10^{-2}	4.8×10^{-2}

[a]See the text and Appendix E for details of the derivation of combined risk estimates.

For oral exposure to tPCP and aPCP (pure PCP), the recommended slope factor is 4×10^{-1} (mg/kg-day)$^{-1}$. This slope factor should not be used with exposures >0.3 mg/kg-day (the POD for the site with the greatest response for tPCP-exposed male mice), because above this point, the slope factor may not approximate the observed dose-response relationship adequately.

For oral exposure to EC-7, the recommended slope factor is 2×10^{-1} (mg/kg-day)$^{-1}$. This slope factor should not be used with exposures >1 mg/kg-day (the POD for the site with the greatest response for EC-7-exposed male mice), because above this point, the slope factor may not approximate the observed dose-response relationship adequately.

Concerning the carcinogenicity of PCP alone, the impurities in the test materials and whether they contribute to the carcinogenicity associated with PCP were considered. Limited quantitative information is available on the carcinogenic potential of the impurities in the formulations of PCP (tPCP and EC-7) tested by NTP (1989). Based on the NTP (1989) calculations, the tPCP formulation is comprised of approximately 90% PCP, 4% TCP, 6% chlorohydroxydiphenyl ethers, and trace amounts of chlorinated dibenzodioxins and dibenzofurans. The EC-7 formulation is comprised of approximately 91% PCP and 9% TCP. The oral slope factor of 4×10^{-1} (mg/kg-day)$^{-1}$ for tPCP may be associated with cancer risk from both PCP and its impurities. Available information addressing carcinogenicity of the impurities varies widely, from a slope factor for hexachlorodibenzodioxins (U.S. EPA, 1988) to no

information regarding the carcinogenicity for most of the impurities. Hexachlorodibenzodioxins comprise 0.001% of tPCP and 0.00002% of EC-7, about a 50-fold difference. The most common impurity in both formulations, TCP, at 3.8% in tPCP and 9.4% in EC-7, shows some evidence of carcinogenicity (see Section 4.1). Although the available data do not support a quantitative risk estimate for TCP, the difference in potencies between the two formulations (if there truly is one) does not suggest a role for TCP, since the difference in potencies is in the opposite direction to the relative amounts of TCP in each formulation.

Estimation of bounding conditions may help in considering the possible impact of the impurities. First, if any carcinogenic risk associated with each set of impurities is negligible relative to that from PCP alone, then in order to use the estimated slope factor for a PCP-only exposure, the slope factor should be adjusted to reflect that the exposure levels in the bioassay were not completely PCP. That is, the slope factor would be multiplied by 1/purity, or 1/0.9 = 1.1, an increase of 10%, because both formulations were approximately 90% PCP.

On the other hand, if the carcinogenic activity of the impurities is not negligible, then the estimated risk attributable to PCP should be reduced. Starting with hexachlorodibenzodioxins, the slope factor was estimated at 6×10^3 $(mg/kg\text{-}day)^{-1}$ (U.S. EPA, 1988[2]). For an exposure level of 1 mg/kg-day of tPCP, there would be 0.00001 mg/kg-day of hexachlorodibenzodioxins, for an estimated lifetime upper bound extra risk of 6×10^{-2}, about sevenfold lower than the estimated lifetime risk using the slope factor for tPCP (4×10^{-1}). Note that about seven impurities are present in tPCP at higher levels than hexachlorodibenzodioxins. Similarly, at 1 mg/kg-day of EC-7, there would be 2×10^{-7} mg/kg-day of hexachlorodibenzodioxins, for an estimated lifetime upper bound extra risk of 1.2×10^{-3}, about 160-fold lower than the estimated lifetime risk using the slope factor for EC-7 (2×10^{-1}). Also note that about five other chlorinated phenols, dioxins, and furans are present in EC-7 at higher levels than the hexachlorodibenzodioxins. These risk comparisons are only approximate, but in view of the other related chemicals present in these formulations without carcinogen assessments they suggest that the slope factors estimated from tPCP and EC-7 data are more relevant for exposures to those formulations, and less relevant for PCP alone or in mixtures other than tPCP and EC-7. However, based on either low toxicity or the presence of minute quantities, the chlorinated dibenzodioxins and dibenzofurans may contribute only slightly to the cancer risk associated with tPCP.

Comparison of the two formulations identifies a common contaminant, TCP. It is unlikely, based on the quantities present in both formulations of PCP, that TCP is largely

[2]The reported slope factor for hexachlorodibenzodioxins was a geometric mean of the slope factors for male mice and female rats: female rat = 3.5×10^3 per mg/kg-day, male mouse = 1.1×10^4 per mg/kg-day. Using the more sensitive response, and adjusting for the current interspecies scaling factor based on $BW^{3/4}$ rather than $BW^{2/3}$ (by multiplying by $(BW_a/BW_h)^{0.33}/(BW_a/BW_h)^{0.25} = 0.083/0.152 = 0.54$), an approximate slope factor for comparison with the PCP slope factors is given by 1.1×10^4 per mg/kg-day x $0.54 \approx 6 \times 10^3$ per mg/kg-day, essentially the same as the reported slope factor for hexachlorodibenzodioxins.

responsible for the difference in the oral slope factors for tPCP and EC-7. The assumption that TCP minimally contributes to the estimated cancer risk for EC-7 indicates that the oral slope factor of 2×10^{-1} (mg/kg-day)$^{-1}$ underestimates the risk associated with aPCP. It is possible that the hydroxydiphenyl ether contaminants are responsible for the difference in cancer potency between tPCP and EC-7; however, given the lack of information on these ethers contaminants, their potential contribution to the carcinogenicity of tPCP cannot be characterized.

In summary, the presence of contaminants in the formulations of PCP tested by NTP (1989) (i.e., tPCP and EC-7) could have contributed to the carcinogenicity of the formulations. Whether these contaminants resulted in an over- or underestimation of the potency of PCP alone cannot be determined. Therefore, the risk associated with tPCP is considered an estimate of the cancer risk associated with aPCP, and the recommended oral slope factor of 4×10^{-1} (mg/kg-day)$^{-1}$ is considered representative of the cancer risk associated with PCP alone.

An inhalation unit risk was not derived in this assessment. Data on the carcinogenicity of the compound via the inhalation route is unavailable, and route-to-route extrapolation was not possible due to the lack of a PBPK model.

5.4.5. Uncertainties in Cancer Risk Values

As in most risk assessments, extrapolation of the available experimental data for PCP to estimate potential cancer risk in human populations introduces uncertainty in the risk estimation. Several types of uncertainty may be considered quantitatively, whereas others can only be addressed qualitatively. Thus, an overall integrated quantitative uncertainty analysis cannot be developed. Major sources of uncertainty in the cancer assessment for PCP are summarized below and in Table 5-7.

Table 5-7. Summary of uncertainties in the PCP cancer risk assessment

Consideration/ approach	Impact on oral slope factor	Decision	Justification
Overall carcinogenic potential	Slope factor could ↓ by ~1.4-fold if based on most sensitive site only	Combined risk, across sites thought to be independent	Basing risk on one site underestimates overall risk when multiple tumor types occur.
Human relevance of male mouse tumor data	Human risk could ↓ or ↑, depending on relative sensitivity	Liver and adrenal gland tumors in male mice are relevant to human exposure	There are no MOA data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. The carcinogenic response occurs across species. PCP is a multi-site carcinogen, although direct site concordance is generally not assumed (U.S. EPA, 2005a); consistent with this view, some human tumor types are not found in rodents.
Bioassay	Alternatives could ↑ or ↓ slope factor by an unknown extent	NTP study	Alternative bioassays were unavailable.
Dose metric	Alternatives could ↑ or ↓ slope factor by an unknown extent	Used administered exposure	Experimental evidence supports a role for metabolism in toxicity, but actual responsible metabolites are not clearly identified.
Low-dose extrapolation procedure	Departure from EPA's *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ slope factor an unknown extent	Multistage model to determine POD, linear low-dose extrapolation from POD (default approach)	Available MOA data do not inform selection of dose-response model; the linear approach is applied in the absence of support for an alternative.
Cross-species scaling	Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by BW] or ↑ twofold [scaling by $BW^{2/3}$])	$BW^{3/4}$ (default approach)	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks.
Statistical uncertainty at POD	↓ slope factor 1.4-fold if a central tendency estimate (i.e., BMD)MLE used rather than lower bound on POD	BMDL (default approach for calculating reasonable upper bound slope factor)	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure.
Human population variability in metabolism and response/sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive.

Overall carcinogenic potential. Considering the multiple tumor types and sites observed in the mice exposed to PCP, the estimation of risk based on only one tumor type/site, even if the most sensitive, may underestimate the overall carcinogenic potential of PCP. An approach based on counts of animals with one or more tumors is expected to underestimate overall risk when tumor types occur independently (NRC, 1994). The MOAs of the liver, adrenal gland, and circulatory system tumors are unknown, so it cannot be verified whether or not these tumors develop independently with PCP exposure. (Note that within sites, adenomas and carcinomas were not assumed to be independent.) The NRC (1994) recommended a simulation approach for combining the risk estimates from each separate tumor type in order to derive the distribution of the BMD for the combined risk of liver, adrenal gland, or circulatory system tumors observed in male and female mice with oral exposure to PCP. A bootstrap analysis (Efron and Tibshirani, 1993) was implemented for these data. For male mice, the overall unit risk was approximately 1.4-fold higher than that from liver tumors alone. If there is some dependency between the sites considered, then the overall carcinogenic potential would be somewhat reduced.

Relevance to humans. The relevance of the MOA of liver tumor induction to humans was considered in Section 4.7.3. There is some evidence in humans (sawmill workers) for hepatic cancer associated with PCP exposure (Demers et al., 2006). The experimental animal literature indicates that PCP induces liver tumors in both male and female mice exposed to two formulations of PCP. Data are limited and preclude the characterization of the MOA by which PCP exerts its carcinogenic effect in the mouse model. Oxidative stress may play a role in the carcinogenicity of PCP observed in mice. Indicators of oxidative stress that were observed in animal studies with PCP have also been identified in human cancers.

The MOA for the adrenal gland tumors (pheochromocytomas and malignant pheochromocytomas) in mice is unknown. In humans, pheochromocytomas are rare catecholamine-producing neuroendocrine tumors that are usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Lehnert et al., 2004; Edstrom Elder et al., 2003; Goldstein et al., 1999). Hereditary factors in humans have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004).

Bioassay selection. The study by NTP (1989) was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes of $B6C3F_1$ mice with 50 animals/sex/dose group, which is typical for carcinogenicity studies. Test animals were allocated among two dose levels of tPCP and three dose levels of EC-7 and an untreated control group for each formulation. Animals were observed twice daily and examined weekly (for 12–13 weeks) for body weight and monthly for feed consumption. Animals were necropsied and all organs and tissues were examined grossly and microscopically for histopathological lesions for a full set of toxicological endpoints in both sexes. Alternative bioassays for quantitative analysis were unavailable. Overall responses across the sexes of the two grades of PCP were similarly

robust, although the responses tended to be greater in those animals treated with tPCP than those treated with EC-7.

Choice of species/gender. The oral slope factor for PCP was quantified using the tumor incidence data for male mice, which were judged to be more sensitive than female mice to the carcinogenicity of PCP. The male rat tumor incidence data, while demonstrating some evidence of carcinogenicity, were not utilized for deriving low-dose quantitative risk estimates. The responses of increased incidence of mesothelioma and nasal squamous cell carcinoma in male rats were lower than those of the mice (NTP, 1989) at a greater exposure level, suggesting greater sensitivity of the mice. Moreover, the toxicological database for PCP studies in rodents has shown the mouse model, rather than the rat, to be a more sensitive model of PCP hepatotoxicity. Although the NTP (1999) bioassay in rats administered aPCP reported mesotheliomas and nasal squamous cell carcinomas, the tumors occurred in male rats of multiple dose groups, but only in the high dose (1-year exposure) was the tumor incidence statistically significant. The lack of a significant dose-response trend in the rat data and the observation of consistently greater sensitivity to PCP in mice, rather than rats, led to the use of the mouse data, specifically the male mouse data (relatively most sensitive), for the derivation of the slope factor. Consequently, dose-response modeling was not carried out with the rat tumor data.

Dose metric. PCP is metabolized to hydroquinone and benzoquinone metabolites; however, it is unknown whether a metabolite or some combination of parent compound and metabolites is responsible for the observed toxicity of PCP. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric provides an unbiased estimate of carcinogenicity. On the other hand, if this is not the correct dose metric, then the impact on the slope factor is unknown.

Choice of low-dose extrapolation approach. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. A linear low-dose extrapolation approach was used as a default to estimate human carcinogenic risk associated with PCP exposure due to the limited availability of data to determine the mode of carcinogenic action of PCP. The extent to which the overall uncertainty in low-dose risk estimation could be reduced if the MOA for PCP were known is of interest, but the MOA is not known.

Etiologically different tumor types were not combined across sites prior to modeling, in order to allow for the possibility that different tumor types can have different dose-response relationships because of varying time courses or other underlying mechanisms or factors. The human equivalent oral slope factors estimated from the tumor sites with statistically significant increases ranged from 0.017 to 0.29 per mg/kg-day, a range less than two orders of magnitude, with the greater risk coming from the male mice tPCP data.

However, given the multiplicity of tumor sites, basing the oral slope factor on one tumor site may underestimate the carcinogenic potential of PCP. Following the recommendations of the National Research Council (NRC 1994) and the EPA's *Guidelines for Carcinogen Risk*

Assessment (U.S. EPA, 2005a) an approach based on combining the risk estimates from each separate tumor type was used. Total carcinogenic risk was estimated using a bootstrap analysis (Efron and Tibshirani, 1993; see Section 5.3) to derive the distribution of the BMD for the combined risk of liver and adrenal gland tumors observed in male mice and the combined risk of liver, adrenal gland, and circulatory system tumors observed in female mice with oral exposure to PCP. Note that this estimate of overall risk describes the risk of developing any combination of the tumor types considered, not just the risk of developing all three simultaneously. The highest combined risk observed, similar to the individual cancer risk estimates, was in tPCP-exposed male mice. The 95% UCL on the combined risk for male mice that developed liver and/or adrenal gland tumors was 4.0×10^{-1} $(mg/kg\text{-}day)^{-1}$, which is about 38% higher than the 2.9×10^{-1} $(mg/kg\text{-}day)^{-1}$ cancer slope factor estimated from liver tumors only in tPCP-exposed male mice.

Choice of model. All risk assessments involve uncertainty, as study data are extrapolated to make inferences about potential effects in humans from environmental exposure. The largest sources of uncertainty in the PCP cancer risk estimates are in determining which formulation to use, interspecies extrapolation, and low-dose extrapolation. There are no human data from which to estimate human cancer risk; therefore, the risk estimate must rely on data from studies of mice exposed to levels greater than would occur from environmental exposures.

Without human cancer data or better mechanistic data, the relevance of the rodent cancer results to humans is uncertain. The occurrence of increased incidences of liver, adrenal gland, and circulatory system tumors in male and female mice exposed to tPCP and nasal squamous cell carcinoma, and mesothelioma in male rats exposed to aPCP from the oral route of exposure suggests that PCP is potentially carcinogenic to humans as well. However, the lack of concordance in tumor sites between the two rodent species makes it more difficult to quantitatively estimate human cancer risk.

Regarding low-dose extrapolation, in the absence of mechanistic data for biologically based low-dose modeling or mechanistic evidence to inform the low-dose extrapolation (see the discussion at the beginning of Section 5.4.3), a linear low-dose extrapolation was carried out from the $BMDL_{10}$. It is expected that this approach provides an upper bound on low-dose cancer risk for humans. The true low-dose risks cannot be known without additional data.

With respect to uncertainties in the dose-response modeling, the two-step approach of modeling only in the observable range (U.S. EPA, 2005a) and extrapolating from a POD in the observable range is designed in part to minimize model dependence. Furthermore, the multistage model used provided an adequate fit to all the datasets. The ratio of the BMD_{10} values to the $BMDL_{10}$ values give some indication of the uncertainties in the dose-response modeling. The ratio between BMDs and BMDLs is typically less than 2 when modeling cancer data (i.e., NTP or other bioassay data with about 50 animals per group). This ratio characterizes the experimental variability inherent in the data. For the tumor sites evaluated for PCP, this ratio

was 1.8 or less, indicating that the estimated risk is not influenced by any unusual variability relative to other assessments. No additional uncertainty is added to the assessment by estimating combined risks reflecting multiple sites. Each combined estimate is a statistically rigorous restatement of the statistical uncertainty associated with each risk estimate derived for individual sites.

Cross-species scaling. An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between mice and humans, consistent with the 2005 *Guidelines for Carcinogen Risk Assessment* (US EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Human population variability. Neither the extent of interindividual variability in PCP metabolism nor human variability in response to PCP has been characterized. Factors that could contribute to a range of human response to PCP include variations in CYP450 levels because of age-related differences or other factors (e.g., exposure to other chemicals that induce or inhibit microsomal enzymes), nutritional status, alcohol consumption, or the presence of underlying disease that could alter metabolism of PCP or antioxidant protection systems. Incomplete understanding of the potential differences in metabolism and susceptibility across exposed human populations represents a major source of uncertainty.

5.4.6. Previous Cancer Assessment

The previous cancer assessment, posted to the IRIS database in March 1991, included an oral slope factor of 1.2×10^{-1} $(mg/kg\text{-}day)^{-1}$. While also based on the NTP (1989) study that currently serves as the basis for the quantitative cancer assessment, the previous oral slope factor was derived using the pooled incidence of tumors in female mice (now thought to underestimate total risk), the linearized multistage procedure, a cross-species scaling factor based on $BW^{2/3}$ (resulting in a twofold higher risk than current methods), and a geometric mean of the slope factors associated with each formulation of PCP, tPCP, and EC-7 (tending toward the lower slope factor of those estimated). The incidence of tumors in the female mice, rather than the males, was used to derive an oral slope factor because hemangiomas and hemangiosarcomas were observed in females. The male mice did not exhibit a significant increase in incidence of hemangiomas and hemagiosarcomas. The hemangiosarcomas were judged to be the tumor of greatest concern because they are morphologically related to known fatal human cancers that are induced by xenobiotics. Based on a preference for the data on hemangiosarcomas and because some male groups experienced significant early loss (observed in male controls in the tPCP study and in male mice in the mid-dose group in the EC-7 study, although the current analysis has shown a lack of significant effect resulting from the early loss in these groups), only the female mice were used in the quantitative risk assessment.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

6.1.1. Noncancer

PCP is a nonflammable, noncorrosive chemical that was first registered in the United States in 1936 as a wood preservative to prevent decay from fungal organisms and insect damage. It was widely used as a biocide and could also be found in ropes, paints, adhesives, canvas, insulation, and brick walls. After use was restricted in 1984, PCP applications were limited to utilization in industrial areas, including utility poles, cross arms, railroad cross-ties, wooden pilings, fence posts, and lumber/timbers for construction. Currently, products containing PCP remain registered for wood preservation, and utility poles and cross arms represent approximately 92% of all uses for PCP-treated lumber.

During manufacture of PCP, the chemical is contaminated with impurities that consist of several congeners of the chlorophenols, chlorinated dibenzo-p-dioxins, and chlorinated dibenzofurans. Of the chlorinated dibenzo-p-dioxin and dibenzofuran contaminants, the higher chlorinated congeners are predominantly found as impurities within technical grades of PCP (approximately 90% purity). Use of the aPCP first requires a purification process to remove the contaminants that are simultaneously created during the manufacturing of PCP.

Instances of PCP poisoning have been documented, indicating the potentially severe consequences of acute, high-dose exposures. Few studies have examined the effects of the lower exposures that occurred in occupational settings or through residential or environmental sources. Many of the available studies are relatively small (<50 participants) (Peper et al., 1999; Triebig et al., 1987; Klemmer et al., 1980; Begley et al., 1977) or may not be representative of the exposed population (Gerhard et al., 1999; Walls et al., 1998). Despite these limitations, there are indications of specific types of neurobehavioral effects seen with chronic exposure to PCP in non-occupational settings (Peper et al., 1999). A larger study of 293 former sawmill workers in New Zealand also suggests neuropsychological effects and respiratory diseases (McLean et al., 2009b). In addition, the results from a large nested cohort study of reproductive outcomes in offspring of sawmill workers (Dimich-Ward et al., 1996) indicate that specific types of birth defects warrant additional research.

The toxicity of PCP in orally exposed animals was investigated in numerous studies in experimental animals. These studies indicate that PCP is toxic to the liver. In chronic studies in rats and dogs, liver toxicity was characterized primarily by increased incidence of chronic inflammation, cytoplasmic vacuolization, pigmentation, and hepatocellular necrosis as well as changes in liver weight (NTP, 1999; Mecler, 1996; Schwetz et al., 1978). Liver toxicity in mice was exhibited as necrosis, cytomegaly, chronic active inflammation, pigmentation, and bile duct

lesions (NTP, 1989). The increased severity of liver toxicity observed in mice versus rats could be based in part on differences in biotransformation of PCP (Lin et al., 1997), but it is also noted that in the mouse studies, the PCP test material contained higher concentrations of chlorinated dibenzo-p-dioxin or dibenzofuran contaminants, which could contribute to the severity of the liver response. Liver toxicity in the dog (Mecler, 1996) was similar to that of the mouse, but the doses inducing toxicity were lower than those in the mouse (i.e., 1.5 mg/kg-day in the dog versus 17–18 mg/kg-day in the mouse). Studies using domestic or farm animals showed that pigs, but not cattle, exhibited similar liver toxicity as that observed in mice. Pigment deposition was also observed in the proximal convoluted tubules in the kidneys of rats (NTP, 1999). Developmental toxicity studies (Welsh et al., 1987; Schwetz et al., 1974a) indicated toxic effects in offspring at dose levels below those producing maternal toxicity. Studies in mink indicate some reproductive effects following exposure to PCP (Cook et al., 1997). The spleen weights of mice (NTP, 1989), rats (Bernard et al., 2002), and cattle (Hughes et al., 1985) were decreased following exposure to PCP.

Disruption of thyroid homeostasis has been observed following the administration of PCP. Several studies have reported decreased serum T_4 and T_3 levels in rats (Jekat et al., 1994) and cattle (Hughes et al., 1985; McConnell et al., 1980). Decreases in serum T_4 have been observed in ram and ewe lambs (Beard et al., 1999a, b), mature ewes (Rawlings et al., 1998), and mink (Beard and Rawlings, 1998) after administration of PCP. TSH was unaffected by treatment with 1 mg/kg-day PCP in calves (Hughes et al., 1985) and sheep (Beard et al., 1999b). However, Jekat et al. (1994) reported a decrease in TSH accompanying the decrease in T_4 levels in rats administered 3 mg/kg-day tPCP and aPCP. Considering that TSH acts on the thyroid to control production of T_4, the concurrent decrease in TSH is in contrast to the expected TSH response to a decrease in T_4 (TSH is generally expected to increase in response to a decrease in T_4), which led Jekat et al. (1994) to suggest that this was due to interference with thyroid hormone regulation at the hypothalamic/pituitary level and possibly increased peripheral thyroid hormone metabolism. However, the available data do not allow for determination of the mechanism involved in the effects on T_3, T_4, and TSH following exposure to PCP. The effect of PCP on thyroid hormone homeostasis has been attributed to PCP and not to contaminants. Changes in thyroid hormones have been associated with effects (i.e., delayed myelination, neuronal proliferation, and synapse formation) on neurons. Considering that thyroid hormones may play a role in neurodevelopmental processes, the disruption of thyroid homeostasis that has been observed with PCP indicates a potential concern for critical period of development of the nervous system (CalEPA, 2006). However, the downstream effects associated with PCP and decreased T_4 levels have not been explored.

Studies examining the immunotoxic effects of PCP showed that the humoral response and complement activity in mice were impaired by tPCP, but not by aPCP, when administered to adult animals (NTP, 1989; Holsapple et al., 1987; Kerkvliet et al., 1985a, b; 1982a). However,

treatment of mice with aPCP from the time of conception to 13 weeks of age resulted in impaired humoral and cell-mediated immunity (Exon and Koller, 1983), suggesting that PCP, and not just the contaminants, induce immunotoxicity. Human studies showed that immune response was impaired in patients who had blood PCP levels >10 μg/L and in particular in those whose levels were >20 μg/L (Daniel et al., 1995; McConnachie and Zahalsky, 1991). Based on the limited available information, immunotoxic effects of PCP may be elicited, in part, through the presence of the dioxin/furan contaminants within PCP.

In vitro neurotoxicity studies showed that PCP causes a dose-dependent irreversible reduction in endplate potential at the neuromuscular junction and interferes with axonal conduction in the sciatic nerve from the toad (Montoya and Quevedo, 1990; Montoya et al., 1988). An NTP (1989) study in mice showed only decreased motor activity in rotarod performance in male rats treated with tPCP for 5 weeks and increases in motor activity and startle response in females receiving purified and tPCP for 26 weeks. Another in vivo study showed that treatment of rats with PCP for up to 14 weeks caused biochemical changes in the rat brain (Savolainen and Pekari, 1979). The most definitive study showed that rats receiving PCP in drinking water for at least 90 days had marked morphological changes in sciatic nerves (Villena et al., 1992).

Elevated blood sugar levels (considered minor by Demidenko, 1969) and increases in organ weights were observed in rats and rabbits exposed to 21–29 mg/m^3 PCP by inhalation for 4 months (Ning et al., 1984; Demidenko, 1969). Additional effects included anemia, leukocytosis, eosinophilia, hyperglycemia, and dystrophic processes in the liver. Minor effects were noted on the liver, cholinesterase activity, and blood sugar effects of animals exposed to 2.97 mg/m^3 (calculated as 0.3 mg/kg-day PCP by Kunde and Böhme, [1978]), a dose that is lower than the lowest NOAELs (1 mg/kg-day) observed in animals orally exposed to 28.9 mg/m^3 PCP (Demidenko, 1969). Ning et al. (1984) reported significant increases in organ weights (lung, liver, kidney, and adrenal glands), serum γ-globulin, and blood-glucose levels at 21.4 mg/m^3.

Studies examining the mutagenicity of PCP have shown that in a variety of test systems, PCP is nonmutagenic, with the exception of one study (Gopalaswamy and Nair, 1992) in which PCP exhibited a positive response for mutagenicity in the Ames salmonella assay. In contrast to data on PCP, data for the TCpHQ metabolite of PCP show positive mutagenic effects in CHO cells (Jansson and Jansson, 1991; Carstens et al., 1990; Ehrlich, 1990), an increase in micronuclei using V79 cells (Jansson and Jansson, 1992), covalent binding to DNA (Witte et al., 2000, 1985), and induction of DNA SSBs (Witte et al., 1985).

6.1.2. Cancer

The available epidemiologic studies support an association between PCP exposure and development of specific cancers: non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, and liver cancer (limited evidence). These studies used PCP-specific exposure

assessment and in some cases, additional assessment of other chlorophenols and potential contaminants. PCP preparations are produced with methods that allow for the formation of contaminants, and degradation products occur naturally in most formulations. However, these contaminants are unlikely to spuriously produce the observed associations seen in the epidemiologic studies, given the difference in the patterns of cancer risk seen in studies of dioxins compared with the studies of PCP, and the relative strengths of the effects of different chemicals (PCP, other chlorophenols, dioxins, and furans) in the studies that examined more than one of these chemicals. It should be noted that in the epidemiological studies examining the cancer risk associated with exposure to PCP, exposures occurred predominantly via the inhalation and dermal routes.

Animal studies with PCP show evidence of adrenal medullary and hepatocellular tumors in male and female mice, hemangiosarcomas and hemangiomas in female mice, and nasal squamous cell carcinomas and mesotheliomas in male rats. Two well-conducted studies provide data for the carcinogenicity of PCP via the oral route in laboratory animals: one study in $B6C3F_1$ mice (NTP, 1989) and another study in F344 rats (NTP, 1999). Two formulations of PCP, tPCP and EC-7, were carcinogenic in the mouse. Hepatocellular adenomas/carcinomas and adrenal medullary pheochromocytomas developed in male mice treated with tPCP or EC-7, and hepatocellular adenomas/carcinomas and hemangiosarcomas developed in female mice treated with tPCP or EC-7 and adrenal medullary pheochromocytomas developed in female mice treated with EC-7.

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), PCP is characterized as "likely to be carcinogenic to humans" by all routes of exposure.

6.2. DOSE RESPONSE

6.2.1. Noncancer—Oral Exposure

The most sensitive endpoints identified for effects of PCP by oral exposure relate to liver toxicity in the chronic gelatin capsule study Mecler (1996) in beagle dogs. Mecler (1996) was selected for the derivation of the oral RfD. This study was conducted in accordance with good laboratory practice guidelines valid at that time and included both sexes of beagle dogs, four animals per sex and dose group, and three dose groups plus controls (0, 1.5, 3.5, and 6.5 mg/kg-day). The study reported multiple toxic endpoints, including changes in absolute and relative organ weights, changes in hematological parameters, and histopathologic outcomes. Hepatotoxicity characterized by dose-related increases in incidence and severity of hepatic lesions (including liver pigmentation, cytoplasmic vacuolation, chronic inflammation, and the appearance of dark, discolored livers) accompanied by significant increases in absolute (in females only) and relative liver weight, and serum activity of ALT and ALP in dogs was considered the critical effect. Another target of PCP toxicity following oral exposure considered in the selection of the critical effect was the developing organism. Studies in experimental

animals found that PCP exposure during gestation can produce prenatal loss, skeletal variations, visceral malformations, decreased fetal weight, and delayed puberty; these doses also produced toxic effects in the dams. However, PCP doses associated with liver toxicity were lower than those associated with developmental toxicity.

Dose-response data of Mecler (1996) were evaluated by using the NOAEL/LOAEL approach with an increase in the incidence of hepatic effects identified as the critical effect. The POD was 1.5 mg/kg-day, the LOAEL. A composite UF of 300 was applied to derive the oral RfD of 5×10^{-3} mg/kg-day. The composite UF of 300 consists of an interspecies UF of 10 for extrapolation from animals to humans, an intraspecies UF of 10 to adjust for sensitive human subpopulations, and a UF of 3 to account for the use of a LOAEL instead of a NOAEL.

Confidence in the principal study, Mecler (1996), is medium. The 52-week study in beagle dogs is an unpublished, Office of Pollution, Prevention and Toxic Substances (OPPTS) guideline study that used three dose groups plus a control and collected interim data at 13, 26, 39 weeks. The study is limited by the use of relatively small group sizes (4 dogs/sex/dose). Because the incidence of two of the key liver effects (i.e., hepatocellular pigmentation in males and females and chronic inflammation in males) increased from 0% in the controls to 100% in the lowest dose tested, and remained at 100% in both the mid- and high-dose groups, the study provided limited resolution of the dose-response curve at low doses. However, liver effects observed in this study (i.e., the critical effect for the RfD) are well-supported by other oral subchronic and chronic studies. PCP also induced toxicity in reproductive and immunological studies, but at doses higher than those used in the principal study. Confidence in the database is high because the database includes acute, short-term, subchronic, and chronic toxicity studies and developmental and multigenerational reproductive toxicity studies in multiple species, and carcinogenicity studies in two species. Overall confidence in the RfD is medium.

6.2.2. Cancer

The NTP (1989) mouse study was selected for dose-response assessment based on statistically significant increased incidence of hepatocellular adenomas and carcinomas and adrenal pheochromocytomas and malignant pheochromocytomas in male and female mice and hemangiomas and hemangiosarcomas (in liver and spleen) in female mice. The study was used for development of an oral slope factor. This was a well-designed study, conducted in both sexes of $B6C3F_1$ mice with two formulations of PCP (tPCP and EC-7) and with 50 mice/sex/dose. Test animals were allocated among two dose levels for tPCP and three dose levels for EC-7 and untreated control groups for each formulation. Animals were observed twice daily and examined weekly (for 12–13 weeks) and then monthly for body weight and monthly for feed consumption. Animals were necropsied and all organs and tissues were examined grossly and microscopically for histopathological lesions for a full set of toxicological endpoints in both sexes. Tumor

incidences were elevated with increasing exposure level at multiple sites in both sexes, including the liver, adrenal gland, and circulatory system.

The male F344 rat tumor incidence data (NTP, 1999), while demonstrating some evidence of carcinogenicity, were not utilized for deriving low-dose quantitative risk estimates, based on evidence of greater sensitivity of the mice to PCP.

A linear approach was applied in the dose-response assessment for PCP, in which the MOA is uncertain, consistent with U.S. EPA's (2005a) *Guidelines for Carcinogen Risk Assessment*. The guidelines recommend the use of a linear extrapolation as a default approach when the available data are insufficient to establish a MOA for a tumor site. As discussed in Section 4.7.3, the mechanism leading to the formation of liver, adrenal, and circulatory tumors in mice following PCP ingestion is unknown. There is some evidence of oxidative damage to cells and DNA adducts from prominent reactive metabolites, and some evidence of cytotoxicity observed in animal and in vitro studies; however, these data do not allow for the identification of key events or support a mode of carcinogenic action. Therefore, a linear extrapolation was used to derive the cancer slope factor for ingested PCP.

Increased incidence of hepatocellular adenomas and carcinomas, benign and malignant adrenal medullary tumors, and hemangiomas and hemangiosarcomas in a 2-year mice bioassay (NTP, 1989) served as the basis for the oral cancer dose-response analysis. A multistage model using linear extrapolation from the POD (combined risk estimates based on increased incidence of both hepatocellular and adrenal gland tumors in male mice) was performed to derive an oral slope factor of 4×10^{-1} $(mg/kg\text{-}day)^{-1}$ for PCP. The recommended slope factor should not be used with exposures >0.3 mg/kg-day (POD for the site with the greatest response for tPCP-exposed male mice), because above this point, the slope factor may not approximate the observed dose-response relationship adequately.

Extrapolation of the experimental data to estimate potential cancer risk in human populations introduces uncertainty in the risk estimation for PCP. Uncertainty can be considered quantitatively; however, some uncertainty can only be addressed qualitatively. For this reason, an overall integrated quantitative uncertainty analysis cannot be developed. However, a major uncertainty considered was the observation of multiple tumor types and sites in the mice exposed to PCP. Risk estimated using only one tumor type/site, even if the most sensitive, may underestimate the overall carcinogenic potential of PCP. Therefore, an upper bound on combined risk was derived in order to gain some understanding of the overall risk resulting from tumors occurring at multiple sites. A bootstrap analysis (Efron and Tibshirani, 1993) was used to derive the distribution of the BMD for the combined risk of liver and adrenal gland tumors observed in male rats with oral exposure to PCP. A simulated incidence level was generated for each exposure group using a binomial distribution with probability of success estimated by a Bayesian estimate of probability. Each simulated data set was modeled using the multistage model in the same manner as was done for the individual risks associated with the liver, adrenal

gland, and circulatory system tumors. The 5th percentile from the distribution of combined BMDs was used to estimate the BMDL corresponding to an extra risk of 1% for any of the three tumor sites. The results of combining risks across sites within datasets are shown in Table 5-6. The highest combined risk observed, similar to the individual cancer risk estimates, was in tPCP-exposed male mice. The 95% UCL on the combined risk for animals that developed liver and/or adrenal gland tumors was 4.0×10^{-1} (mg/kg-day)$^{-1}$, which is about 38% higher than the 2.9×10^{-1} (mg/kg-day)$^{-1}$ cancer slope factor estimated from liver tumors only in tPCP-exposed male mice. The risk estimates for the tPCP-exposed males and females tend to be higher than those for the EC-7-exposed animals, by approximately twofold for both the central tendency and upper bound estimates.

A biologically-based model was not supported by the available data; therefore, a multistage model was the preferred model. The multistage model can accommodate a wide variety of dose-response shapes and provides consistency with previous quantitative dose-response assessments for cancer. Linear low-dose extrapolation from a POD determined by an empirical fit of tumor data has been judged to lead to plausible upper bound risk estimates at low doses for several reasons. However, it is unknown how well this model or the linear low-dose extrapolation predicts low-dose risks for PCP. An adjustment for cross-species scaling ($BW^{3/4}$) was applied to address toxicological equivalence of internal doses between mice and humans based on the assumption that equal risks result from equivalent constant lifetime exposures.

An inhalation unit risk was not derived in this assessment. Data on the carcinogenicity of the compound via the inhalation route is unavailable, and route-to-route extrapolation was not possible due to the lack of a PBPK model. However, it is proposed that PCP is likely to be carcinogenic to humans by the inhalation route since the compound is well-absorbed, and in oral studies induces tumors at sites other than the portal of entry.

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The Toxicological Review of Pentachlorophenol (dated April 2009) has undergone a formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000a). 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 responses to these comments arranged by charge question follow. In many cases the comments of the individual reviewers have been synthesized and paraphrased in development of Appendix A. An external peer-review meeting was held August 4, 2009. EPA also received scientific comments from the public. These comments and EPA's responses are included in a separate section of this appendix.

EXTERNAL PEER REVIEWER COMMENTS

The reviewers made several editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further.

A. General Charge Questions

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazards?

Comments: Two reviewers considered the document to be well written. One of the reviewers commented that the document is logical and clear, but could be more concise (i.e., less repetitive). One reviewer found the document to provide an accurate, clear, and objective presentation of the studies. This reviewer did note some editorial and grammatical errors. Another reviewer commented that the presentation of the toxicological and epidemiological data was very logical, clear, and concise. One reviewer considered the review of the literature to be thorough and comprehensive and presented in a logical manner. This reviewer stated that the weight of evidence of pentachlorophenol toxicity to be objectively analyzed. One reviewer considered the organization of the Toxicological Review to be cumbersome. This reviewer did not find the science regarding the mode of action to have been adequately evaluated, in particular the failure to have incorporated the initiation/promotion study by Umemura et al. (1999) in the analysis of the mode of action (MOA) for liver tumors.

Response: The content of the Toxicological Review is consistent with the current outline for IRIS toxicological reviews. The document was reviewed and edited to improve clarity and reduce repetition. The study by Umemura et al. (1999) is described in detail in Section 4.2.4.1.1, Initiation/promotion Studies; discussion of the findings of this study was added to Section 4.7.2.2., Animal Cancer Evidence from Oral Exposure.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of PCP.

Comments: Two reviewers were not aware of any additional studies that should be included in the assessment. Two reviewers identified the following published studies for consideration:

Folch, J; Yeste-Velasco, M; Alvira, D; et al. (2009) Evaluation of pathways involved in pentachlorophenol-induced apoptosis in rat neurons. Neurotoxicology 30:451-8.

McLean, D; Eng, A; Walls, C; et al. (2009a) Serum dioxin levels in former New Zealand sawmill workers twenty years after exposure to pentachlorophenol (PCP) ceased. Chemosphere 74:962-7.

McLean, D; Eng, A; Dryson, E; et al. (2009b) Morbidity in former sawmill workers exposed to pentachlorophenol (PCP): a cross-sectional study in New Zealand. Am J Ind Med 52:271-81.

Mirabelli, MC; Hoppin, JA; Tolbert, PE; et al. (2000) Occupational exposure to chlorophenol and the risk of nasal and nasopharyngeal cancers among US men aged 30 to 60. Am J Ind Med 37:532-41.

Orton, F; Lutz, I; Kloas, W; and Routledge, EJ. (2009) Endocrine disrupting effects of herbicides and pentachlorophenol: in vitro and in vivo evidence. Environ Sci Technol 43:2144-50.

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Zhu, BZ and Shan, GQ. (2009) Potential mechanism for pentachlorophenol-induced carcinogenicity: a novel mechanism for metal-independent production of hydroxyl radicals. Chem Res Toxicol 22:969–977.

One of these reviewer identified a new NIOSH epidemiological study that was said to provide evidence for an association between exposure to PCP and a risk of non-Hodgkin’s lymphoma However, this reviewer noted that the study is currently unpublished (Ruder et al., unpublished).

One reviewer noted that the findings in Umemura et al. (1999) comparing rat and mouse liver effects that were discussed in Section 4.2.4.1.1 should have also been incorporated into the discussions on MOA (Sections 4.5 and 4.7.3).

Response: Summaries of the McLean et al. cohort studies of serum dioxin levels (2009a) and morbidity (e.g., respiratory and neurological effects, 2009b) in former New Zealand sawmill

workers exposed to PCP were added to Sections 4.1.2.2 and 4.1.2.3. These studies are considerably larger than any other studies examining these types of effects.

A reviewer also suggested adding the Mirabelli et al. (2000) study of case-control study of nasal and nasopharyngeal cancers in relation to chlorophenol exposure. This study is a parallel study to the Hoppin et al. (1998) case-control study of soft tissue sarcoma; that is, these two studies were conducted using the same study design and exposure assessment. A full description of the Hoppin et al. (1998) study was not included in the Toxicological Review because they presented data only for a combined exposure (e.g., chlorophenols, or chlorophenols and phenoxy herbicides). However, this study along with several other studies that presented data only for a combined exposure (e.g., chlorophenols, or chlorophenols and phenoxy herbicides) are noted in Section 4.1.1.1. Mirabelli et al. (2000) and 't Mannetje et al. (2005) have been added to the studies listed in this section. These studies present information for a combined category of chlorophenols, for five occupational exposure categories that were the basis for estimating chlorophenol exposure (cutting oils, leather work, saw/pulp/planning mill, shoe/leather dust, and wood preserving chemicals), and for the occupational exposure category described as plywood/fiberboard/particleboard and wood/saw dust. Because the authors did not include a discussion of the relative contribution of pentachlorophenol to each of these categories, these studies were not considered directly useful for an assessment of pentachlorophenol hazard. The unpublished NIOSH study was not included in the current assessment because it is not currently part of the peer-reviewed literature.

Relevant information from the other studies identified by the reviewers were added to the Toxicological Review.

As noted in response to a comment under General Charge Question #1, further discussion of the initiation/promotion study by Umemura et al. (1999) was added to Section 4.7.2.2., Animal Cancer Evidence from Oral Exposure.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of PCP.

Comments: Reviewers offered suggestions for additional research to address the data gaps for PCP, most of which focused on the need for further elucidation of a cancer MOA and the development of an RfC. Specific research recommendations included the following:

- Epidemiologic studies focusing on quantitative exposure assessment
- Studies of PCP metabolism
- Development of toxicokinetic models for route-to-route extrapolation to allow for the development of an RfC
- Inhalation studies to support development of an RfC

- Studies in the low-dose range, focusing on endpoints pertinent to endocrine disruption and neurological effects
- A study of aPCP that could further define the dose response to allow for benchmark dose modeling
- Comparison of the quinone metabolites of PCP in the liver nuclei of dogs, mice, and rats
- Further research on the cancer MOA to reduce uncertainty in the cancer assessment, with one reviewer suggesting molecular techniques such as microarray analysis, and another suggesting that genotoxicity testing, specifically the comet assay or nucleotide post-labeling, be performed in target organs for PCP-induced carcinogenicity
- Dermal toxicity studies

Response: EPA agrees that additional research in the areas recommended by the peer reviewers would increase the confidence in the PCP database for future toxicological assessments of this chemical.

4. Please comment on the identification and characterization of sources of uncertainty in Sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Comments: Two reviewers specifically commented that the choices and assumptions made in the discussion of uncertainty were transparently and objectively described. One of these reviewers specifically noted that the section on uncertainty was concise and thoughtful. The other reviewer indicated that the impact of the uncertainties identified in the assessment were adequately presented.

Several reviewers offered suggestions to more completely characterize the sources of uncertainty associated with the PCP database. One reviewer offered comments on a specific uncertainty factor; these comments are summarized and addressed in response to RfD Charge Question #3.

Two reviewers suggested that PODs for the cancer assessment be estimated using other models in BMDS to provide quantitative information regarding the degree of uncertainty/sensitivity associated with the choice of the low-dose extrapolation procedure. One reviewer also thought that uncertainty related to tumor site concordance (i.e., that the quantitative cancer assessment is based on liver, adrenal and circulatory system cancers, whereas the most commonly reported association in the epidemiologic literature is lymphomas) should be addressed. One reviewer questioned the conclusion in the Toxicological Review that no additional uncertainty is added to the assessment by estimating combined risks reflecting

multiple tumor sites and the assumption that the carcinogenesis process is completely independent across tumor sites. This reviewer suggested discussion of this assumption and the choice of prior distribution as a source of uncertainty.

One reviewer identified uncertainties in the principal study associated with a study duration that was shorter than other chronic studies of PCP and small numbers of animals tested. Another reviewer suggested that a discussion of the uncertainty inherent in comparing effects from different compositions of PCP would be helpful.

Response: With respect to the comment regarding tumor site concordance, EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state that "… agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans." Therefore, the lack of site concordance between animals and humans is not considered to be a significant uncertainty in the assessment.

Identification of the potential for impurities to influence the toxicity of the PCP formation tested by Mecler (1996) (90.6% PCP) was added as an additional area of uncertainty in Section 5.3.

Comments on the selection of the principal study, EPA's procedure for estimating combined risks from multiple tumor sites and the assumption of independence of the carcinogenesis process across tumor sites, and use of other models in BMDS to better evaluate the sensitivity of the selected analysis are addressed in response to comments under RfD Charge Question #1 and Cancer Charge Questions #2 and 6.

Chemical-Specific Charge Questions:

B. Oral Reference Dose (RfD) for Pentachlorophenol

1. A 1-year oral study in dogs by Mecler (1996) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Has this study been transparently and objectively described in the document? Are the criteria and rationale for this selection transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Comments: Two reviewers agreed that the selection of the Mecler (1996) study was scientifically justified. Four of the reviewers noted that the study was transparently and objectively described in the document. Two reviewers commented that the selection of the Mecler (1996) study as the principal study was the appropriate study on which to base the RfD since this study identified

hepatotoxicity at the lowest dose tested in the available studies. One reviewer questioned whether the study by Kimbrough and Linder (1978), rather than Mecler (1996), resulted in the lowest RfD, and noted a discrepancy between the figure and table presented in Section 5.1.4. One reviewer commented that the nature of the liver pigmentation described in the Mecler (1996) study needed clarification. This reviewer also noted that there was no discussion of absorption, distribution, metabolism, and excretion (ADME) in dogs.

Response: The study by Mecler (1996) represents the most sensitive chronic oral study for PCP. Table 5-1 and Figure 5-1 were corrected to show that the study by Kimbrough and Linder (1978) yields a candidate RfD that is higher that the RfD derived from Mecler (1996). Discussion of uncertainty associated with the Kimbrough and Linder (1978) study in Section 5.1.1 was revised. Text was added to the summary of the Mecler (1996) study in Section 4.2.1.3 to better describe the nature of the liver pigmentation as described by the study authors. Information on ADME of PCP in dogs is not available.

2. An increase in hepatic effects (characterized by a dose-related increase in the incidence of hepatocellular pigmentation, cytoplasmic vacuolation, chronic inflammation, and severely discolored livers; statistically significant increases in absolute (females only) and relative liver weights, and serum enzyme activity) as reported by Mecler (1996) was selected as the critical effect for the RfD because these effects are considered by EPA to be indicative of hepatocellular injury. Please comment on whether the rationale for the selection of this critical effect is scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Comments: All of the reviewers agreed with the selection of the critical effect. One reviewer, while stating that use of the Mecler (1996) study and subclinical hepatic effects as the principal study and critical effect was acceptable, questioned whether necrosis as reported in the study by Kimbrough and Linder (1978) would yield a lower RfD and thus serve as a more appropriate critical effect for derivation of the RfD. One reviewer commented that they agreed that based on the available mode of action data, PCP is capable of inducing hepatocellular injury. This reviewer stated that they would have selected a newer study utilizing lower doses if they were available. The reviewer also stated that if newer studies were completed, they expected that endocrine and neurological endpoints would be the most sensitive.

Response: The description of the study by Kimbrough and Linder (1978) incorrectly indicated that liver necrosis was observed in rats exposed to the highest dose tested of tPCP. In addition,

necrosis was used to describe the effects observed at the LOAEL in Table 5-1 and Figure 5-1. In fact, necrosis was never observed in this study. The text has been corrected to reflect the actual findings.

No studies on neurological or endocrine-related endpoints are available for PCP. The study by Mecler (1996) represents the most sensitive chronic oral study for PCP.

3. The hepatotoxic data and a NOAEL/LOAEL approach were used to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether this is the best approach for determining the POD. Has it been transparently and objectively described? Please identify and provide rationales for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comments: Three reviewers specifically agreed with the application of the NOAEL/LOAEL approach to derive the POD. Two reviewers recommended a clearer, more convincing rationale for not conducting BMD modeling. One of these reviewers noted that a 100% response in dose groups, the absence of a NOAEL, and small group sizes should not preclude BMD modeling. One reviewer suggest that it would be possible to conduct BMD modeling on the data from the Kimbrough and Linder (1978) study. In addition, one reviewer mentioned that it would be of interest to compare RfD values derived from studies that produced a NOAEL value at the lowest dose tested to those derived from the LOAEL identified by Mecler (1996) in order to see whether the values were similar, thus providing further support for the POD chosen for deriving the RfD.

Response: In Mecler (1996), the incidence of hepatocellular pigmentation in males and females and chronic inflammation in males increased from 0% in the controls to 100% in the low-dose group, both the mid- and high-dose groups also had 100% responses. These data were not amenable to BMD modeling, as none of the dose-response models in BMDS can adequately accommodate this steep increase in response. Thus, the NOAEL/LOAEL approach was employed to identify the POD. Text was added to Section 5.1.2, Methods of Analysis—NOAEL/LOAEL Approach to clearly articulate the rationale behind selecting the LOAEL/NOAEL approach.

Figures 5-1 and 5-2 provide graphical comparisons of candidate PODs that represent NOAELs and LOAELs from alternative studies and data sets that were considered as the basis for the PCP RfD.

4. The RfD is based on toxic effects observed in dogs (Mecler, 1996) administered a technical grade formulation of PCP (90.9% purity). Considering the toxicological database for PCP is largely comprised of studies that utilized similar formulations, as well as

commercial and analytical (pure) formulations, please provide comments with regard to whether the use of data based on animal exposure to a technical grade PCP formulation of this purity is the best approach and can be considered representative of pure PCP. If not, please identify and provide the rationale for any alternative data sets, and the sufficiency of such data sets, to support derivation of the RfD.

Comments: All five peer reviewers agreed that technical grade PCP can be considered representative of pure PCP.

Response: No response necessary.

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document? If changes to the uncertainty factors are proposed, please identify and provide a rationale(s). Please comment specifically on the following uncertainty factor:

- **An uncertainty factor of 3 was applied in deriving the RfD to account for the use of a LOAEL rather than a NOAEL as the POD.**

Comments: Two reviewers thought that the selection of a UF_L of 3 was appropriate. Three other reviewers questioned the characterization of the hepatic effects observed in the Mecler (1996) study as mild, and did not find the rationale for using a UF_L of 3 rather than 10 to be adequately justified.

Response: The discussion of the UF_L in Section 5.1.3 was revised to provide better justification for the selection of a UF of 3 to extrapolation from LOAEL to a NOAEL.

C. Inhalation Reference Concentration (RfC) for Pentachlorophenol

1. An RfC was not derived due to the lack of available studies to characterize the health effects associated with pentachlorophenol administered via the inhalation route. Are there available data that might support development of an RfC for pentachlorophenol?

Comments: All of the peer reviewers agreed that available data do not support the development of an RfC.

Response: No response necessary.

D. Carcinogenicity of Pentachlorophenol

1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that pentachlorophenol is "likely to be carcinogenic" to humans. Please comment on the cancer weight of evidence characterization. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described? Do the available data for liver, adrenal gland, and circulatory system tumors in mice and nasal tumors and mesotheliomas in rats support the conclusion that PCP is a likely human carcinogen?

Comments: Four of the five reviewers agreed that the classification of PCP as "likely to be carcinogenic" to humans was appropriate based on tumor incidence in animal studies and epidemiological data. One of these reviewers thought that the rationale for selecting "likely to be carcinogenic" over other descriptors should have been explicitly stated. One reviewer stated that only a descriptor of "possibly carcinogenic to humans" could be supported by animal tumor findings in the absence of a MOA of established relevance to humans. This reviewer was unable to locate the WOE descriptor.

Response: Supporting data for the descriptor of "likely to be carcinogenic to humans" may include human studies demonstrating a plausible (but not definitively causal) association between exposure and cancer and positive studies in animals in more than one species, sex, strain, site, or exposure route. Section 4.7.1. of the Toxicological Review presents the data supporting the descriptor of "likely to be carcinogenic to humans" for PCP. PCP is "likely to be carcinogenic to humans" based on positive studies in more than one species, sex, and site along with supporting data demonstrating a plausible (but not definitively causal) association between human exposure and cancer. Specifically, that database includes (1) evidence of carcinogenicity from oral studies in male mice exhibiting hepatocellular adenomas and carcinomas, pheochromocytomas and malignant pheochromocytomas, and in female mice exhibiting hepatocellular adenomas and carcinomas, pheochromocytomas and malignant pheochromocytomas, and hemangiomas and hemangiosarcomas (NTP, 1989); (2) some evidence of carcinogenicity from oral studies in male rats exhibiting malignant mesotheliomas and nasal squamous cell carcinomas (Chhabra et al., 1999; NTP, 1999); (3) evidence from human epidemiologic studies showing increased risks of non-Hodgkin's lymphoma and multiple myeloma, some evidence of soft tissue sarcoma, and limited evidence of liver cancer associated with PCP exposure (Demers et al., 2006; Hardell et al., 1995, 1994; Kogevinas et al., 1995); and (4) positive evidence of hepatocellular tumor-promoting activity (Umemura et al., 2003a, b, 1999) and lymphoma and skin-adenoma promoting activity in mice (Chang et al., 2003).

Section 2.5 of EPA's 2005 *Guidelines for Carcinogen Risk Assessment,* presents a description of the weight of evidence narrative and evaluation of the types of data supporting the weight of evidence descriptor selection. In general, the weight of evidence descriptor is selected based on the complete evaluation of the data and includes a summary of potential modes of action and how they support the overall conclusions and weight of evidence descriptor. The WOE descriptor for PCP is presented in Section 4.7.1, Summary of Overall Weight of Evidence, under the Evaluation of Carcinogenicity, along with the rationale for selecting the descriptor.

2. A quantitative oral cancer assessment has been derived for PCP. Do the data support an estimation of a cancer slope factor for PCP? Please comment on the scientific justification for deriving a quantitative cancer assessment. Has the rationale and scientific justification for quantitation been transparently and objectively described?

Comments: All five reviewers agreed that the available data are sufficient for deriving a cancer slope factor. Four of the reviewers commented that the rationale and justification for the quantitative assessment was adequately described in the document. One reviewer provided no comment regarding the rationale and justification for the quantitative assessment. One reviewer commented that a comparison of different modeling approaches to better evaluate the sensitivity of the selected analysis would be useful.

Response: In the absence of sufficient data or understanding to develop a robust, biologically-based cancer model, a single preferred curve-fitting model is applied (U.S. EPA, 2005). Many different curve-fitting models have been developed, and those that fit the observed data reasonably well may lead to several-fold differences in estimated risk at the lower end of the observed range; however, goodness-of-fit to the experimental observations is not by itself an effective means of discriminating among models that adequately fit the data. As noted in Section 5.4.3, the multistage model in the BMDS suite of models is used by EPA as the preferred model for cancer dose-response modeling because it is thought to reflect the multistage process of cancer and it fits a broad array of dose-response patterns. Furthermore, use of this model across the vast majority of quantitative cancer assessments provides a measure of consistency across different cancer assessments. For these reasons, other modeling approaches were not presented in the Toxicological Review.

3. A two-year oral cancer bioassay (NTP, 1989) in mice was selected as the principal study for the development of an oral slope factor. Please comment on the appropriateness of the selection of the principal study. Has the rationale for this choice been transparently and objectively described?

Comments: Three of the reviewers agreed that the NTP bioassay (1989) was the appropriate choice. One reviewer commented that the presentation of the development of the slope factor was well presented with objectivity and good transparency. This reviewer noted that selection of the principal study with multiple tumor sites allowed for estimation of a statistically appropriate upper bound on total risk (combined slope factor), which described the risk of developing any combination of tumor types considered. One reviewer recommended that the possibility of a threshold (or nonlinear approach) for cancer be considered since the MOA for PCP involves promoting action. One reviewer commented that mouse liver carcinogenicity is species specific and that justification for the selection of mouse liver tumor data should be provided.

Response: The MOA for PCP-induced carcinogenicity is unknown. As discussed in Sections 4.7.3 and 5.4.3, the available data indicate that multiple modes of carcinogenic action are possible, but none have been defined sufficiently (e.g., key events for carcinogenicity, temporal relationships) to inform the shape of the dose-response curve at low doses. Therefore, there are insufficient data to establish significant biological support for a nonlinear approach.

The available data for PCP demonstrate that the mouse is more sensitive to PCP-induced carcinogenicity than the rat. In addition, in the absence of MOA data informing human relevancy of mouse liver tumors, data from the mouse, specifically the combined risk of liver tumors and adrenal gland pheochromocytomas, was used to derive the PCP cancer slope factor. The uncertainties associated with the selection of these tumor data sets for derivation of the slope factor as well as justification for their selection is discussed in Sections 4.7.3 and 5.4.5.

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4. Data on the mode of action (MOA) of carcinogenicity of PCP were considered. Several hypothesized MOAs were evaluated within the Toxicological Review and EPA reached the conclusion that a MOA(s) could not be supported for any tumor types observed in animal models. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively described. Please comment on data available for PCP that may provide significant biological support for a MOA beyond what has been described in the Toxicological Review.

Comments: Four of the five reviewers agreed that the scientific evidence supports the conclusion that a MOA could not be established. One reviewer commented that the MOA data were not adequately discussed. Specifically, the reviewer commented on the lack of consideration of studies finding mouse liver tumor promotion but a lack of initiation following PCP exposure. This reviewer questioned why rat liver was not discussed as a target of PCP metabolites as shown by Lin et al. (1977) and why the data for oxidative damage were considered too limited to consider it as a possible MOA. This reviewer also suggested that further justification be

provided to support the conclusion that oxidative stress-induced DNA damage is thought to be related to the formation of electrophilic metabolites of PCP that are capable of binding to DNA.

Response: In Section 4.7.3, Mode-of-Action Information, the available studies on PCP-induced liver tumor promotion, the species differences in liver tumors between mice and rats, and the oxidative damage induced by PCP metabolites are all discussed and considered. However, there are several other responses to PCP exposure that can have promoting effects and could be involved in the MOA, including necrosis and chronic inflammation leading to reparative cell proliferation/regeneration and interference with GJIC, as well as other types of genotoxic damage, including DNA adduct formation. Therefore, the precise MOA for the carcinogenic effects of PCP could not be definitively determined. Clarification regarding the conclusions related to oxidative stress-induced DNA damage have been made to document.

5. Increased incidence of tumors in male and female B6C3F$_1$ mice was observed following administration of two formulations of PCP [technical grade PCP and EC-7 (a commercial grade of PCP)] that contain various chlorophenol and chlorinated dibenzodioxin and dibenzofuran contaminants. The carcinogenic contributions of PCP versus those of contaminants have been described qualitatively and to a limited extent quantitatively within the document. The cancer assessment is based on the data sets resulting from exposure to two different formulations that are approximately 90% PCP, with the assumption that carcinogenic contributions from the contaminants are minimal. Please comment on the scientific justification and transparency of this analysis. Please comment on whether these are the appropriate data sets on which to base the cancer risk estimate and, if not, please identify and provide the rationale for any alternative data sets, and the sufficiency of such data sets, to support estimation of cancer risk.

Comments: The reviewers generally agreed with EPA's assumption that the contribution of chemical contaminants to the carcinogenic response of tPCP is minimal. Two reviewers suggested that EPA consider pooling the findings from the NTP study for tPCP with the EC-7 findings on the basis that the two formulations have similar PCP content and the carcinogenicity for both is attributable to PCP, and assuming these studies do not have significantly different tumor results. One of these reviewers noted that it is possible that differences in slope factors for tPCP and EC-7 are due to random variability in the experimental responses, rather than some difference in the underlying formulations. The reviewer considered this view supported by the fairly similar BMD values for tPCP and EC-7. One reviewer considered the approach for rescaling the slope factors by 1/purity to be justified, but noted that this purity rescaling was not applied in estimating the slope factors for aPCP. One of these reviewers observed that the mouse

liver promotion study of Umemura et al. (1999) performed with aPCP supports the interpretation that the liver effects are due to aPCP.

Response: The tumor incidence in mice exposed to tPCP or EC-7 for 2 years in the NTP (1989) bioassays differed quantitatively. For example, the incidence of hepatocellular adenoma was almost twofold higher in tPCP-exposed male mice compared to EC-7-exposed mice; similarly, the incidence of adenoma or carcinoma was 1.8-fold higher in tPCP-exposed male mice compared to EC-7-exposed mice. Whether these differences reflect random variability in tumor response or differences resulting from differences in the composition of the impurities in the two PCP formulations is uncertain. Given this uncertainty, the two data sets were modeled separately (see discussion in Section 5.4.2).

Section 5.4.4 presents a discussion of the potential impact of impurities on the value of the oral slope factor for aPCP derived from data for tPCP. If the carcinogenic risk associated with impurities is negligible relative to that from PCP alone, scaling by 1/purity (or 1/0.9), which would increase the slope factor by 10%, is appropriate. On the other hand, if the carcinogenic activity of impurities is not negligible, the PCP slope factor should be reduced. In the absence of information to establish the impact of impurities on the oral cancer potency, neither an adjustment to reduce or increase the slope factor was applied. If scaling by 1/purity was applied, an increase in the estimated slope factor by 10% would not change the value of the PCP slope factor when rounded to one significant figure [i.e., $(4.0 \times 10^{-1}) \times 0.1 = 4.4 \times 10^{-1}$ or, rounded to one significant figure, 4×10^{-1}]. Text was added to Section 5.4.4 to clarify why the oral slope factor was not adjusted to account for impurities in the tPCP.

6. Data on tumors in the liver and adrenal gland in B6C3F$_1$ male mice administered technical PCP were used to estimate the oral cancer slope factor. Please comment on the estimation of a statistically appropriate upper bound on total risk (combined slope factor), which described the risk of developing any combination of tumor types considered. Please comment on the scientific justification and transparency of the analysis for combining these data to derive the oral cancer slope factor. Please comment on the use of data in male mice exposed to technical PCP for a cancer risk estimate for both technical and analytical PCP.

Comments: Four reviewers agreed that an approach for deriving a slope factor for technical PCP involving a combined risk across tumor types was justified; the fifth reviewer did not offer a response to this charge question. One of the four reviewers questioned aspects of the combined risk analysis, suggesting that a parametric bootstrap technique would be advantageous, i.e., preclude the use of a uniform Bayesian prior and having confidence limits more comparable with BMDS. One reviewer asked whether the assumption of independence across cancer sites could be tested by further analyzing historical tumor data from the NTP database.

Response: The Bayesian choice, which is actually a binomial estimate of the probability, not a full-blown Bayesian analysis was utilized to prevent the re-sampling probability from being 0 or 1. For a control group incidence of 0, as is the case with both male and female tPCP data (see Table D-1), the parametric estimate of the control probability is 0. Hence the reviewer's suggestion would likely somewhat underestimate variability. In an analysis not shown in the document, EPA did compare BMDs and BMDLs from a bootstrap analysis with BMDS results and found a good correspondence.

Tumor-type associations among individual animals in 62 B6C3F_1 mouse studies and 61 F344 rat studies from the NTP database were evaluated by Bogen and Seilkop (1993). The NRC (1994) considered this evaluation and reported that tumor-type occurrences in NTP bioassays were in most cases nearly independent, and that the few departures that were detected were small.

PUBLIC COMMENTS

Comment: One commenter expressed their support for the LOAEL to NOAEL UF of 3, noting that a UF of 3 was applied to the same study (Mecler, 1996) used to derive the chronic RfD in EPA's Office of Pesticide Programs' 2008 Registration Eligibility Decision (RED) document for PCP. This commenter observed that questions have been raised about the relevance of the liver effects at the low dose (1.5 mg/kg-day) and that selection of this dose as the LOAEL already reflects a conservative choice.

Response: The LOAEL to NOAEL UF of 3 was retained. The justification for this selection in Section 5.1.3 was clarified.

Comment: A commenter disagreed with EPA's conclusion that the mode(s) of action for liver tumors has not been defined sufficiently to inform low-dose extrapolation for estimation of PCP carcinogenicity. The commenter stated that chronic oxidative stress leading to generation of reactive oxygen species and liver toxicity (as demonstrated by dose-related increases in apurinic and apyrimidinic sites, 8-OHdG, and single-strand breaks in DNA) are strongly supported as the mechanism for tumor induction, and that oxidative stress occurred only at high PCP exposures. The commenter also observed that species differences in susceptibility to PCP could be explained by differences in the rate of glutathione depletion, with conjugation of reactive oxygen species with glutathione serving as a protective mechanism against reactive oxygen species-induced toxicity. Because glutathione depletion was not expected to occur in humans, the commenter considered humans not to be susceptible to glutathione depletion at current exposures to PCP. The commenter concluded that neither oxidative stress nor glutathione depletion were

likely to occur in humans at levels to which humans are exposed to PCP. As such, the commenter argued that the MOA for PCP is expected to be nonlinear and that the current cancer assessment for PCP should be replaced by a nonlinear assessment.

Response: As discussed in Section 4.7.3, EPA determined that the MOA for PCP induction of liver tumors is unknown. While EPA agrees that evidence supports oxidative stress as playing a role in tumor induction, the mechanisms involved and extent of contribution are not fully understood. Rather, the available data indicate that multiple modes of carcinogenic action are possible, but that none have been defined sufficiently (e.g., key events, temporal relationships) to inform low-dose extrapolation. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that linear extrapolation of cancer risk be used as a default approach where the weight of evidence evaluation is insufficient to establish the MOA for a tumor site. Consistent with this guidance, EPA does not consider application of a nonlinear extrapolation approach to be supported.

Comment: A commenter identified a 2009 paper on hydroxyl radical formation (Zhu and Shan, 2009) that was not included in the Toxicological Review.

Response: A summary of this paper was added to the Toxicological Review.

Comment: A commenter stated that (1) because of the higher incidence of liver tumors with tPCP than Dowicide EC-7, the dioxin and furan contaminants that are at higher concentrations in tPCP may contribute greatly to the increased incidence of liver tumors with tPCP, and (2) it is possible that the liver tumors induced by Dowicide EC-7 could be related to the contaminants and not PCP alone.

Response: In Section 5.4.4, specific consideration was given to the possible contributions of impurities in tPCP and EC-7 on tumor response. While uncertainties associated with impurities are acknowledged, EPA concluded that the oral slope factor of 4×10^{-1} $(mg/kg\text{-}day)^{-1}$ can be considered representative of the cancer risk associated with PCP alone.

Comment: A commenter stated that there is no evidence that mouse pheochromocytomas are relevant to the assessment of human risk because (1) the incidence of malignant pheochromocytomas was not statistically significantly increased in males or females, (2) benign tumors are not considered relevant to humans, and (3) there is no epidemiology evidence to support that pheochromocytomas can be induced in humans under any conditions (Elmore et al., 2009). The commenter stated that recommendations of the 1990 Science Advisory Board (SAB) supported this opinion.

Response: No studies were identified to determine a mode of action for PCP-induced tumors of the adrenal gland. Pheochromocytomas are catecholamine-producing neuroendocrine tumors. The relevance of rodent pheochromocytomas as a model for human cancer risk has been the subject of discussion in the scientific literature (e.g., Greim et al., 2009; Powers et al., 2008). In humans, pheochromocytomas are rare and usually benign, but may also present as or develop into a malignancy (Eisenhofer et al., 2004; Lehnert et al., 2004; Elder et al., 2003; Goldstein et al., 1999; Salmenkivi et al., 2004; Tischler et al., 1996). Rates of malignant transformation of 10% (Salmenkivi et al., 2004; Sweeney, 2005) to approximately 36% have been reported (Ohta et al., 2006). Hereditary factors in humans have been identified as important in the development of pheochromocytomas (Eisenhofer et al., 2004). Pheochromocytomas are more common in laboratory rats, though evidence suggests that certain rat pheochromocytomas may have similarity to human pheochromocytomas (Powers et al., 2009). Furthermore, mechanisms of action inducing pheochromocytomas in rats are expected to occur in humans as well (Greim et al., 2009). Therefore, in the absence of information indicating otherwise, adrenal gland tumors in rodents are considered relevant to humans. No studies were identified to determine a mode of action for PCP-induced tumors of the adrenal gland. Thus, the mode of action for pheochromocytomas observed following oral exposure to PCP is unknown.

Parallels between pheochromocytomas in the mouse and humans have led investigators to suggest that the mouse might be an appropriate model for human adrenal medullary tumors (Tischler et al., 1996). Like humans, the spontaneous occurrence of pheochromocytomas in the mouse are relatively rare (≤3%; Tischler et al., 2004, 1996), as are metastases. The morphological variability of mouse pheochromocytomas and the morphology of the predominant cells are comparable to those of human pheochromocytomas. An important characteristic of mouse pheochromocytomas is expression of immunoreactive phenylethanolamine-N-methyltransferase (PNMT), the enzyme that produces epinephrine from norepinephrine; human pheochromocytomas are also usually PNMT-positive (Tischler et al., 1996).

Elmore et al. (2009) states that there is no epidemiologic evidence that adrenal medullary proliferative lesions can be induced in humans under any circumstances, but that the rodent tumors express many of the same genes as their human counterparts and are potentially valuable for mechanistic studies of the roles of those genes in tumor biology. No case-control or other studies in humans that evaluated possible associations between pheochromocytomas and environmental agents are available in the published peer-reviewed literature. Thus, while the epidemiological literature does not provide evidence of pheochromocytoma induction by various agents, it appears that no such studies have been performed.

The SAB committee stated that the increased incidence of pheochromocytomas and dose-response pattern was related to PCP exposure. The committee noted that there is disagreement in the interpretation of the meaning of pheochromocytomas in rodents and the diagnoses of these

lesions. The SAB questioned the human relevance of these tumors based on the fact that only benign tumors were observed. However, the committee did not state that pheochromocytomas are not relevant to humans.

Comment: A commenter stated that the MOA for hemangiomas and hemangiosarcomas in female mice is consistent with oxidative stress, and that such a MOA would have a nonlinear dose-response. The commenter specifically cited research on vinyl chloride, a chemical that induces hemangiosarcoma and is metabolized to chloroethylene oxide, a mutagen that induces four adducts that have been shown to be induced by oxidative stress as support for this determination.

Response: In the absence of any mechanistic data specific to the induction of hemangiomas and hemangiosarcomas by PCP, a MOA for this tumor type is unknown. Consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), linear extrapolation of cancer risk was applied to data for hemangiomas and hemangiosarcomas as a default approach where the weight of evidence evaluation was insufficient to establish the MOA.

Comment: A commenter stated that the most likely operative MOA for mesotheliomas of the peritoneal cavity, originating from the tunica vaginalis of the testes in male rats, was oxidative stress. The commenter observed that these tumors were increased in male rats in the stop-exposure component of the NTP (1999) study, but not the 2-year study. The commenter also suggested that another MOA contributing to this tumor type is hormonal imbalance brought about by perturbations of the endocrine system, which is associated with the formation of Leydig tumors of the testes that occur spontaneously at a high incidence in F344/N rats.

Response: The possible role of oxidative stress in PCP carcinogenicity is discussed in Section 4.7.3 and is discussed with respect to mesothelioma by Chhabra et al. (1999). EPA did not identify any literature that addresses PCP induction of mesothelioma via hormonal imbalance. Chhabra et al. (1999) concluded that further studies are needed to fully explain the molecular events leading to mesothelioma formation by PCP. Mesothelioma in the male rat as observed in the stop-exposure study was included in the evaluation of the overall weight of evidence for PCP carcinogenicity, but was not used as the basis for slope factor derivation in this assessment.

Comment: A commenter raised doubts about the nasal squamous cell carcinomas observed in the "stop-exposure" study in the rat (NTP, 1999) because the incidence of nasal tumors was not increased in the full two-year study, and further argued that this tumor was not relevant to humans at current PCP exposure levels. Possible explanations offered for the increased tumor incidence were direct contact of the nasal mucosa membrane with PCP vapor during feeding or

to PCP-containing feed dust, and oxidative damage.

Response: As noted by Chhabra et al. (1999), the nasal effects in the treated rats in the NTP stop-exposure study may have been due to systemic exposure to PCP, direct contact of the nasal mucous membrane with PCP vapor during ingestion of feed, or PCP-containing dust from feed. Studies providing support for PCP-induction of nasal cell carcinomas via direct contact with PCP vapor or dust are not available. Nasal squamous cell carcinomas in the male rat as observed in the stop-exposure study were included in the evaluation of the overall weight of evidence for PCP carcinogenicity, but were not used as the basis for slope factor derivation.

Comment: A commenter suggested that the rat may be a more appropriate animal model than the mouse for assessing risks of PCP to humans for the following reasons: (1) the high spontaneous rate of liver adenomas/carcinomas in male mice, (2) the unusual sensitivity of the B6C3F1 mouse, (3) a "surprisingly" low incidence of liver tumors in concurrent controls in the mouse study, possibly due to low survival of control animals and smaller groups sizes (35 per sex in the control group versus 50 in the treated groups), and (4) contribution of dioxins and furans (at higher levels in tPCP) to the higher liver tumor incidence in mice in the tPCP study. Further, the commenter observed that the incidence of liver tumors was not increased in PCP-exposed F344 rats, which has a lower spontaneous incidence of liver tumors, and that such an increase in the rat would have provided more convincing evidence that PCP is hepatocarcinogenic.

This commenter observed that the incidence of hemangiomas/hemangiocarcinomas was increased only in the female mouse (not in the male mouse or in rats of either sex) and only at the highest doses tested in the studies of tPCP and Dowicide EC-7.

Finally, this commenter stated that data from the rat study may be more relevant because of the greater tendency for glutathione (an important detoxification mechanism of reactive oxygen species) depletion to occur in the mouse than the rat and human and the purity of PCP used in the rat study compared to the mouse studies (99% to 90.4-91%, respectively).

Response: As noted in response to peer review comments under Cancer Charge Question #3, the mouse model has been shown to be more sensitive to PCP carcinogenicity than the rat model. In the absence of MOA data to establish that the mouse model is not relevant to humans, tumor data from the male mouse (specifically the combined risk of liver tumors and adrenal gland pheochromocytomas) was used to derive the PCP cancer slope factor. Uncertainties associated with the selection of this tumor data set for derivation of the slope factor are discussed in Sections 4.7.3 and 5.4.5.

In response to the comment regarding the findings for hemangiosarcomas/ hemangiocarcinomas in female mice, EPA notes that the oral slope factor for PCP is based on male mouse data.

The comment related to the contribution of dioxins and furans to the cancer response observed in mice exposed to tPCP and EC-7 is addressed above.

Comment: A commenter claimed that EPA ignored the recommendations of the 1990 SAB related to the relevance or lack of relevance of tumors in the male and female mouse and with regard to calculation of the oral slope factor, and suggested that disregarding available expert advice can affect the credibility of EPA's risk assessment process.

Response: EPA performed the cancer assessment for PCP consistent with current Agency *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Scientific understandings of cancer and risk assessment practices have evolved in the two decades since the SAB's review of the PCP assessment. It is not unexpected that some of the views and recommendations offered by the SAB in 1990 might differ from current risk assessment practices.

Comment: A commenter cited several issues related to EPA's derivation of the cancer slope factor for PCP, including the following:

(1) A MOA of oxidative stress has been shown for PCP. The commenter stated that oxidative DNA damage is the most common endogenous DNA damage in cells, and that exposure to PCP results in the formation of additional identical adducts. For agents acting through this MOA, a nonlinear approach should be used. To that end, the commenter stated that the draft assessment ignored the EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, under which the preferred method for risk assessment is use of a biologically-based model that incorporates MOA considerations.

(2) A slope factor based derived by combining adrenal and liver tumors is inconsistent with the recommendations of the 1990 SAB.

(3) The slope factor based on data for tPCP is recommended for use with pure PCP, in spite of the fact that EC-7 was indicated to have lower levels of dioxins and furans.

(4) EPA chose to apply defaults of the risk assessment methodology, including the assumption that humans were more sensitive than the most sensitive species (mouse). The commenter reiterated the position related to the lack of relevance of mouse liver tumors, that the benign tumor response in the mouse liver and adrenal are more reflective of an epigenetic or nongenotoxic MOA (proposing as modes of action a sustained increased cellular turnover and hormonal challenge), and that the incidence of hemangiomas/hemangiosarcomas may be increased due to oxidative stress that occurs at high exposures or related to by the contaminants.

Response: Reponses to comments related to the use of a nonlinear analysis, concerns about dioxin and furan impurities in tPCP, and tumor relevance are provided above.

The derivation of an oral slope factor based on the combined risk of liver and adrenal gland tumors is consistent with the recommendations in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). As discussed in Section 5.4.5.1, given the multiplicity of tumors sites associated with PCP exposure, an oral slope factor based on one tumor site may underestimate the carcinogenic potential of PCP.

Comment: A commenter questioned whether the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) had been followed, raising specific questions about the choice of epidemiologic studies that EPA relied on for the determination of the weight of evidence descriptor in the assessment. This commenter stated that "it is unknown (and unexplained) why the IRIS conclusions only relied on four studies (out of the many available)."

Response: EPA evaluated a large number of studies with data pertaining to chlorophenols. Section 4.1 presents summaries of the studies that included specific information relevant to PCP. Although all of these studies were considered in the evaluation, the studies cited in the weight-of-evidence narrative (Section 4.7) were the sawmill worker cohort study of cancer incidence and mortality that included specific evaluation of PCP as well as trichlorophenol (Demers et al., 2006) and the case-control studies with detailed PCP assessment (see Tables 4-3 and 4-4). Cohort studies with non-specific exposure assessment, cohorts set in manufacturing plants, and case-control studies with limited PCP assessment were given little weight in the overall cancer evaluation. The relative strengths and limitations of these sets of studies are described in Section 4.1, and this discussion has been added to the summary in Section 4.7.2.1.

Comment: A commenter also discussed the role that tests of statistical significance should play in the evaluation of the body of evidence, and noted that for at least one of the four key studies relied on by EPA in the cancer weight-of-evidence evaluation that statistical significance was either not considered or minimized.

Response: Statistical significance reflects the magnitude of the observed effect and the precision of the estimate. The statistical power of the study (i.e., the probability of correctly identifying a true effect of a specified size) should also be considered in the evaluation of the data; statistical power is directly related to the size of the study (e.g., number of cases in a case-control study) and prevalence of exposure. A study with a low power or probability of detecting a statistically significant result should not necessarily be interpreted as a "null" or "no effect" study. As noted in the EPA's *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005), other considerations, including the magnitude of the point estimate, the precision of this estimate, and the appropriateness of the statistical test that was used should also be considered. Thus, EPA

concluded that studies in which a statistically significant association (e.g., a risk ratio or odds ratio) is not observed should not necessarily be interpreted as evidence of no effect.

Comment: Referring to the body of research on phenoxy herbicides and chlorophenols, a commenter noted that in some manufacturing scenarios, trichlorophenol rather than PCP was used in the production process. The commenter notes that "only when such studies demonstrate that exposure to PCP was explicitly considered can results be afforded greater weight in a weight of evidence (WOE) analysis."

Response: EPA agrees that studies focusing specifically on PCP should be the basis of the analysis. Accordingly, the criteria used in the selection of studies emphasized the availability of PCP-specific data (see description in Section 4.1.1.1), and relatively little weight was given to the cohort study by Ramlow et al. (1996) set in a PCP manufacturing plant that did not include detailed exposure assessment specific to PCP (see discussion in Section 4.1.1.4.)

Comment: With respect to the case-control study of non-Hodgkin's lymphoma by Hardell et al. (1994), a commenter noted a strong association observed with high-grade exposure to PCP (defined as one or more week continuous exposure or one or more month total exposure) with an odds ratio (OR) of 8.8 (95% CI 3.4, 24), but stated that such a high value for an odds ratio is "not plausible and the wide confidence intervals also cast some doubt on the validity of the findings." The commenter also stated that it is not clear if any of the chlorophenol-exposed subjects were also exposed to the phenoxyacetic acids 2,4-D and 2,4,5-T, and questioned why the authors stated that most subjects had been exposed to 2,4-D and 2,4,5-T when the overall prevalence of this exposure was 47 out of 105 cases and 51 out of 335 controls.

Response: EPA agrees that the imprecision of the estimated association results in considerable uncertainty with respect to whether a threefold, fourfold, eightfold or higher risk was seen in the high PCP exposure group, but the data nonetheless indicate an increased risk. With respect to co-exposure with the phenoxyacetic acids, the data indicate some overlap in these groups (among 105 cases, 35 exposed to chlorophenols, 25 exposed to phenoxyacetic acids, and 47 exposed to either chlorophenols or phenoxyacetic acids; among 355 controls, 35 exposed to chlorophenols, 24 exposed to phenoxyacetic acids, and 51 exposed to either chlorophenols or phenoxyacetic acids). The similarity in these patterns among cases and controls, however, argues against confounding as an explanation for the observed association with PCP. Hardell et al.'s (1994) statement in the results section that "Mostly, a combination of 2,4-D and 2,4,5-T had been used in both occupational and leisure time exposure and the statement in the abstract that "Most cases and controls were exposed to a commercial mixture of 2,4-dichlophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid appear to be based on the data from Table 2 of the paper showing

the breakdown in frequency of specific phenoxyacetic acids, in which only 3 cases and 1 control were exposed only to 2,4-D.

Comment: With respect to the nested case-control study of soft tissue sarcoma (12 cases) and non-Hodgkin's lymphoma (32 cases) within 24 cohort studies conducted in 11 countries by Kogevinas et al. (1995), the commenter summarized the results for chlorophenols and for PCP. The association between any chlorophenol exposure and non-Hodgkin's lymphoma was OR 2.75, 95% CI (0.45, 17.0), and the association between high PCP and non-Hodgkin's lymphoma was OR 4.19, 95% CI (0.59, 29.59). The commenter observed that "[t]o the extent that this study was able to isolate exposure to only chlorophenols or PCP specifically, there was no significant increase in either STS [soft tissue sarcoma] or NHL [non-Hodgkin's lymphoma] associated with exposure. These results suggest that neither exposure to chlorophenols, or to PCP in particular, is associated with increased risk of STS or NHL."

Response: Given the limited number of observed cases of either disease in Kogevinas et al. (1995) and the magnitude of the point estimates for the association between PCP and non-Hodgkin's lymphoma (i.e., a three- or fourfold increased risk), it is not appropriate to characterize the observed results as evidence of no association. In addition, as discussed in Section 4.1.1.3, the different pattern of results seen for the chlorophenols other than PCP and for phenoxy herbicides also suggests relative specificity of effects for PCP.

Comment: A commenter summarized the results of the comparisons between cancer rates among the workers in a cohort study by Demers et al. (2006) and reference rates in British Columbia, Canada and a set of exposure-response analyses using an internal referent group within the cohort. These analyses indicated an association with non-Hodgkin's lymphoma, multiple myeloma and kidney cancer, but not with soft tissue sarcoma.

Response: EPA agrees with the study summary, but also notes the additional evidence concerning liver cancer seen in this cohort study.

Comment: A commenter provided an evaluation of the findings of Hardell et al. (1994, 1995), Kogevinas et al. (1995), and Demers et al. (2006) in terms of strength of association, consistency of association, dose-response relationship, temporality, specificity of association, and biological plausibility. The commenter stated that only statistically significant associations between exposure and outcomes were judged to be relevant and that strength of association refers primarily to size of the relative risk which must reach statistical significance.

Response: Both statistical significance and statistical power should be considered when interpreting results of a study. To dismiss from consideration all results that do not reach statistical significance, particularly those from low-powered studies, would be to dismiss pieces of evidence that should be considered in the weight-of-evidence evaluation. EPA's evaluation of a study or collection of studies is made with consideration of the magnitude of effects, precision of effect estimates, and likelihood of estimates.

Comment: A commenter stated that the Demers et al. (2006) cohort study found no significant association or no association between exposure to all chlorophenols (i.e., pentachlorophenol and trichlorophenol) and non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, and liver cancer.

Response: These statements apply when only the results using the external comparison group, i.e., the standardized incidence ratio and standardized mortality ratio data using the British Columbia population as a referent group (Table 2 of Demers et al., 2006) are evaluated. The data from the internal cohort comparisons are important in the evaluation of the association between exposure to chlorophenols and non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, and liver cancer. Tables 3, 4, 5, 6 and 7 of Demers et al. (2006) present the more extensive analyses developed specifically for PCP and trichlorophenol, rather than combining the exposures into the single category (along with unexposed individuals within the plant) that was used in the analysis with the external comparison group. The use of an internal comparison group reduces the likelihood of potential confounders affecting the results. EPA used these internal exposure-response analyses in the evaluation of the study (see Table 4-2).

Comment: A commenter stated that the strong associations between non-Hodgkin's lymphoma and PCP seen in Hardell et al. (1994) study were too strong and imprecise to be believable. The commenter also stated that the results were more likely due to exposure to 2,4-D and 2,4,5-T.

Response: As noted previously, EPA agrees that the imprecision of the estimated association results in uncertainty regarding the magnitude of the observed risk, but does not consider this imprecision to negate the presence of an increased risk. In addition, there is no indication of a disproportionately higher rate of co-exposure with phenoxyacetic acids among cases compared with controls.

Comment: A commenter provided a reference to a case-control study of non-Hodgkin's lymphoma (Hardell and Eriksson, 1999) that had not been included in the Toxicological Review.

Response: A summary of this study was added to Section 4.1.1; the findings of this study are also included in the discussion in Section 4.7. This case-control study included 404 male cases age ≥25 years diagnosed with non-Hodgkin's lymphoma between 1987 and 1990 in northern Sweden. The association seen with PCP exposure was OR = 1.2 (95% CI 0.7, 1.8). PCP use had been banned in Sweden in 1977, so the exposure time period in relation to timing of diagnosis differs in this study compared with the earlier studies from Sweden.

Comment: With respect to the discussion of "consistency," a commenter stated that consistency refers to the presence of a [statistically] significant association in studies of similarly exposed populations.

Response: EPA views study results as estimates, and evaluates the magnitude and precision of the estimates. EPA does not view study results as dichotomous (i.e., either the presence of absence of statistical significance).

Comment: A commenter discussed the available epidemiological studies of PCP and non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, and liver cancer. The summaries are presented in terms of the presence or absence of a statistically significant association, and a specific disease that was not examined in a study is described as being "not mentioned" by that study (e.g., the commenter states that a case-control study of non-Hodgkin's lymphoma did not mention multiple myeloma). The non-Hodgkin's lymphoma literature is summarized as showing a significant association in Hardell et al. (1994) and no association in Hardell and Eriksson (1999). The commenter concluded that there is a lack of consistency in the association for this endpoint.

Response: Demers et al. (2006) study, which reported an increased incidence and increased mortality of non-Hodgkin's lymphoma (trend p-values = 0.03 for incidence and 0.06 for mortality; an approximate twofold increased risk in the two highest exposure groups). In addition, there is a difference in time period between the two Swedish case-control studies. The earlier study (Hardell et al., 1994), in which strong associations were seen, was conducted in cases diagnosed between 1974 and 1978; the later study was conducted in cases diagnosed between 1987 and 1990). PCP use had been banned in Sweden in 1977, which would be expected to result in a considerably different set of exposure conditions.

Comment: With respect to multiple myeloma, a commenter stated that thestudies cited as the basis for the WOE conclusion did not show a significant association with multiple myeloma.

Response: The Demers et al. (2006) cohort study of sawmill workers shows an exposure-response trend for both incidence (trend p-value = 0.03) and mortality (trend p-value = 0.02). The risk ratios in the highest category of exposure were strong (>4.0), and there was no evidence of similar patterns in the analyses of TCP exposure. None of the other studies cited examined multiple myeloma. The Toxicological Review also described the study by Pearce et al. (1986a) of farming-related exposures and multiple myeloma risk in New Zealand (76 cases, 315 controls drawn from a population cancer registry). This study demonstrated that there was little evidence of an association with the general category of chlorophenol exposure (OR = 1.1, 95% CI 0.4–2.7) and work in a sawmill or timber merchant (OR 1.1, 95% CI 0.5–2.3) and stronger associations were seen with a history of doing fencing work (OR 1.6, 95% CI 0.9–2.7) and jobs that involved potential exposure to chlorophenols at a sawmill or timber merchant (OR 1.4, 95% CI 0.5–3.9). Because of the limited information pertaining specifically to PCP in this study, it was not cited in the weight of evidence summary.

Comment: For soft tissue sarcoma, a commenter stated that the association between exposure to chlorophenols and PCP reported by Hardell et al. (1995) was not replicated in a later study (Hardell and Eriksson, 1999) which was not cited in the Toxicological Review. In addition, the commeter also noted that the study by Kogenias et al. (1995) showed no increase and that soft tissue sarcomas were not mentioned in Hardell et al. (1994). The commenter considered that these findings demonstrated a lack of consistency.

Response: The 1999 study by Hardell and Eriksson is a case-control study of non-Hodgkin's lymphoma, and does not provide any data regarding multiple myeloma. The 1999 Hardell and Eriksson study did not fail to replicate the original finding, but instead did not present data on multiple myelomas. Hardell et al. (1995) is a meta-analysis of four separate studies. It is true that Kogenias et al. (1995) did not show an association between PCP exposure and soft tissue sarcoma risk, but it should also be noted that this analysis was based on only 11 cases.

Comment: A commenter described the results of specific studies categorized disease category in terms of the statistical significance of trend tests. For example, for non-Hodgkin's lymphoma observed in the Demers et al. (2006) study the commenter indicated that the study authors showed a significant dose-response trend with incidence, but not with mortality and significant dose-response trends in the incidence and mortality analyses that were lagged by 10 or 20 years. The commenter also summarized the Demers et al. (2006) data with respect to multiple myeloma by noting "a significant dose-response trend" in the incidence and mortality analyses in the lagged and unlagged data.

Response: The argument that the incidence data show a significant dose-response trend that is not seen in the mortality data rests solely on the statistical significance of the trend test, which is p = 0.03 for the incidence data and 0.06 for the mortality data (see Table 4-2 of the Toxicological Review). EPA considers a more accurate characterization of these data to be that both the incidence and the mortality data, in the lagged and unlagged analyses, provide evidence of exposure-response trends, with approximately a twofold increased risk in the highest two categories of exposure. The attenuation of the exposure-response seen in the highest exposure category is commonly seen in epidemiologic studies of occupational cohorts (Stayner et al., 2003). Further, the characterization of the pattern of response across exposure groups should be based solely on the presence or absence of a test for linear trend that is statistically significant at a specified alpha level. The actual pattern of response should be examined when characterizing the data. For example, in addition to the trend p-value, Demers et al. (2006) observed approximately a fourfold increased risk of multiple myeloma in the highest exposure group (see Table 4-2).

Comment: A commenter stated that none of the studies reported a significant dose-response trend between soft tissue sarcoma and PCP.

Response: The Demers et al. (2006) data suggest an inverse association (lower risk with higher exposure). However, this pattern is based on only 5 cases in the highest three quartiles of exposure, however, and are therefore associated with considerable uncertainty.

Comment: A commenter noted that the Demers et al. (2006) study is the only study with data relating to liver cancer and that the data show no increases in this cancer or dose-response or latency trends with exposure to PCP.

Response: Table 4-2 presents the data for the Demers et al. (2006) study. The internal cohort exposure-response analyses was the primary focus of the Demers et al. (2006) study. As stated above in the previous discussion of non-Hodgkin's lymphoma, an attenuation in the highest exposure group was observed. Specifically, relatively strong associations (i.e., at least a doubling of the risk in almost all of the exposure categories) were observed. EPA concluded that these data do not support the conclusion that there is no evidence of an association with liver cancer.

Comment: A commenter noted inconsistencies in the results reported by Hardell et al. (1994, 1995), Demers et al. (2006), and Kogevinas et al. (1995), and concluded that the criterion for

specificity of association (i.e., a single effect being produced by a particular exposure) was not met.

Response: EPA agrees that the difference in the results among these studies is an important consideration and therefore the summaries of these studies describe these data as providing some evidence of carcinogenicity. In addition, the differences between results among these studies are also described. It is also important to note the important methodological differences in the studies, specifically, that the meta-analysis (Hardell et al., 1995) included 434 cases compared with the 23 observed cases in Demers et al. (2006) and 12 observed cases in Kogevinas et al. (1995).

Comment: A commenter suggested that the data show a lack of site concordance between animal and human studies (i.e., tumors seen in experimental exposure studies in rats or mice correlate with the type of tumors seen in humans). This commenter further noted that hepatocellular carcinoma, adrenal medullary neoplasms, and hemangiosarcomas (a histologic form of soft tissue sarcoma), were seen in the animal studies and thus would be the expected types of cancers that would be seen in epidemiological studies of PCP-exposed populations. The commenter stated that the observation of hemangiosarcomas in rats is in contrast to the overall weight of evidence in human studies suggesting that exposure to PCP is not associated with increased risk of soft tissue sarcoma.

Response: EPA concluded that the human studies provide some evidence of soft tissue sarcoma and limited evidence of liver cancer associated with PCP exposure. In addition, the lack of site concordance between animals and humans does not necessarily support a lack of biological plausibility.

Comment: A commenter maintained that site-specificity is always found in epidemiologic studies of chemical carcinogens. Specifically, that human response to exposures to carcinogens are consistent (i.e., of the same type of nature) and that chemical carcinogens display target organ specificity.

Response: Variability in the type of tumors observed in both human and animal studies is common in studies of carcinogens. Many factors can influence the effect of a carcinogen in human populations, including genetic susceptibility, nutritional status, co-exposures, etc.

Comment: A commenter noted that the summary statement in Section 5 regarding the basis for the cancer weight-of-evidence descriptor as “likely to be carcinogenic to humans” by all routes

of exposure based on inadequate evidence from human studies and adequate evidence from animal studies is inconsistent with the discussion presented in Section 4.7.

Response: The summary statement in Section 5 has been revised to better reflect the discussion in Section 4.7.

APPENDIX B: PHYSIOCHEMICAL DATA FOR PCP AND THE IDENTIFIED TECHNICAL- AND COMMERCIAL-GRADE CONTAMINANTS

Table B-1. Physicochemical data for dioxin contaminants of PCP

General chemical formula	Common name	Vapor pressure (mm Hg)	Water solubility at 25°C (mg/L)	Henry's law constant (atm × m^3/mol)	Log K_{ow}
C_6HCl_5O	PCP	0.00415	14	0.079	–
1,2,3,7,8-PeCDD	Pentachlorodibenzo-p-dioxin	4.4×10^{-10}	0.000118	2.6×10^{-6}	6.64
1,2,3,4,7,8-H_xCDD	HxCDD	3.8×10^{-11}	4.42×10^{-6}	1.7×10^{-5}	7.8
1,2,3,6,7,8-H_xCDD	HxCDD	3.6×10^{-11}	4.42×10^{-6}	1.7×10^{-5}	7.8
1,2,3,7,8,9-H_xCDD	HxCDD	4.9×10^{-11}	4.42×10^{-6}	1.7×10^{-5}	7.8
1,2,3,4,6,7,8-HpCDD	Heptachlorodibenzo-p-dioxin	5.6×10^{-12}	2.4×10^{-6}	1.26×10^{-5}	8.0
1,2,3,4,6,7,8,9-OCDD	OCDD	8.25×10^{-13}	7.4×10^{-8}	6.75×10^{-6}	8.2

Table B-2. Physicochemical data for furan contaminants of PCP

General chemical formula	Common name	Vapor pressure (mm hg)	Water solubility at 25°C (mg/L)	Henry's law constant (atm × m^3/mol)	Log K_{ow}
1,2,3,7,8-PeCDF	Pentachlorodibenzofuran	1.7×10^{-9}	–	–	6.79
2,3,4,7,8-PeCDF	Pentachlorodibenzofuran	2.6×10^{-9}	2.36×10^{-4}	4.98×10^{-6}	6.5
1,2,3,4,7,8-HxCDF	Hexachlorodibenzofuran	2.4×10^{-10}	8.25×10^{-6}	1.43×10^{-5}	7.0
1,2,3,6,7,8-HxCDF	Hexachlorodibenzofuran	2.2×10^{-10}	1.77×10^{-5}	7.31×10^{-6}	7.0
2,3,4,6,7,8-HxCDF	Hexachlorodibenzofuran	2.0×10^{-10}	ND	ND	7.0
1,2,3,4,6,7,8-HpCDF	Heptachlorodibenzofuran	3.5×10^{-11}	1.35×10^{-6}	1.41×10^{-5}	7.4
1,2,3,4,7,8,9-HpCDF	Heptachlorodibenzofuran	1.07×10^{-10}	ND	ND	ND
2,3,4,7,8-PCDF	Pentachlorodibenzofuran	ND	ND	ND	ND
1,2,3,4,6,7,8,9-OCDF	Octachlorodibenzofuran	3.75×10^{-12}	1.16×10^{-6}	1.88×10^{-6}	8.0

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Table B-3. Average daily dose of PCP (mg/kg) and contaminants (µg/kg) to B6C3F$_1$ mice in the 2-year feeding study

PCP/contaminant	Males					Females				
	100 ppm		200 ppm		600 ppm	100 ppm		200 ppm		600 ppm
	tPCP	EC-7	tPCP	EC-7	EC-7	tPCP	EC-7	tPCP	EC-7	EC-7
PCP[a]	18	18	35	37	118	17	17	35	34	114
Trichlorophenol	1.1	0.8	2.3	1.6	4.7	1.1	0.8	2.2	1.5	4.6
TCP	430	1,100	860	2,100	6,300	415	1,000	830	2,000	5,800
HCB	0.6	0.7	1.1	1.5	4.4	0.54	0.7	1.1	1.4	4.2
TCDD	–	–	–	–	–	–	–	–	–	–
HxCDD	0.11	0.002	0.23	0.004	0.01	0.11	0.002	0.22	0.004	0.01
Heptachlorodibenzo-p-dioxin	3.3	0.006	6.7	0.01	0.04	3.2	0.006	6.5	0.01	0.03
OCDD	15.6	0.008	31	0.02	0.05	15.1	0.008	31	0.02	0.05
Pentachlorodibenzofuran	0.016	–	0.03	–	–	0.014	–	0.03	–	–
Hexachlorodibenzofuran	0.11	0.001	0.24	0.003	0.009	0.11	0.001	0.22	0.003	0.008
Heptachlorodibenzofuran	1.0	0.002	2.0	0.003	0.01	1.0	0.002	1.9	0.003	0.01
Octachlorodibenzofuran	0.5	–	1.0	–	–	0.5	–	1.0	–	–
Heptachlorohydroxydiphenyl ether	10	–	20	–	–	10	–	20	–	–
Octachlorohydroxydiphenyl ether	220	–	430	–	–	210	–	420	–	–
Nonachlorohydroxydiphenyl ether	400	–	800	–	–	390	–	780	–	–
Hexachlorohydroxydibenzofuran	20	–	40	–	–	20	–	30	–	–
Heptachlorohydroxydibenzofuran	50	–	110	–	–	50	–	100	–	–

[a]Daily dose in mg/kg body weight.

Source: NTP (1989).

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APPENDIX C: PCP LEVELS IN OCCUPATIONALLY EXPOSED HUMANS

Table C-1. Pentachlorophenol levels in worker and residential populations (with ≥15 individuals per group)

Population, location	Serum or plasma				Urine				Reference
	n	Mean	(Range or SD)	Unit	n	Mean	(Range or SD)	Unit	
Occupationally exposed workers									
Hawaii									Bevenue et al., 1967
worker sample									
exposed - pesticide operators					130	1,802	(3–35,700)	ppb	
nonexposed - other workers					117	40	(ND–1,840)	ppb	
population sample									
occupational exposures					121	465	(3–38,642)	ppb	
no occupational exposures					173	44	(3–570)	ppb	
Hawaii									Klemmer, 1972
exposed - open vat wood treaters	22	3.78	(4.00)	ppm	18	0.95	(1.93)	ppm	
exposed - pressure tank wood treaters	24	1.72	(2.02)	ppm	23	0.27	(0.56)	ppm	
farmers (mixed pesticides exposure)	280	0.25	(0.88)	ppm	210	0.01	(0.05)	ppm	
controls (no occupational exposure)	32	0.32	(1.26)	ppm	32	0.03	(0.18)	ppm	
United Kingdom									Jones et al., 1986
exposed - formulators	29	1.3	(0.4–4.8)	mmol/L	26	39.6	(7.4–300)	nmol/mmol creatinine	
exposed - sprayers	108	6.0	(0.2–29.0)	mmol/L	112	274	(11–1,260)	nmol/mmol creatinine	
exposed - timberyard operators	68	4.8	(0.3–45.0)	mmol/L	54	74.0	(5–655)	nmol/mmol creatinine	
nonexposed - furniture makers	61	0.2	(0.1–0.6)	mmol/L	--		not measured	nmol/mmol creatinine	

Table C-1. Pentachlorophenol levels in occupationally exposed populations (with ≥15 individuals per group)

Population, location	Serum or plasma				Urine				Reference
	n	Unit	Mean	(Range or SD)	n	Unit	Mean	(Range or SD)	
Residential or work site exposure[a]									
United States									Cline et al., 1989
exposed (residential)	123	ppb	420	(39–1.340)	118	ppb	69	(1–340)	
exposed (telephone line workers)	13	ppb	110	(26–260)	143	ppb	3.4	(1–17)	
nonexposed	34	ppb	40	(15–75)	117	ng/mg creatinine	3.1	(1–12)	
Germany									Gerhard et al., 1999
exposed	65	µg/L	35.9	(20.7–133)					
nonexposed	106	µg/L	9.5	(2.8–19.3)					
Germany									Peper et al., 1999
exposed	15	µg/L	43.6	(31.2)					
nonexposed	15	µg/L	11.8	(4.5)					
General population									
United States									Hill et al., 1995
(NHANES[b] III)						µg/L µg/g creatinine	2.5 1.8	(ND[c]–55) (ND–29)	

[a]Residents of homes or workers in work places in which PCP was used as a wood preservative on logs or wood used in the construction of these sites.
[b]NHANES = National Health and Nutrition Examination Survey.
[c]ND = nondetectable.

APPENDIX D: DOSE-RESPONSE MODELING OF CARCINOGENICITY DATA FOR PENTACHLOROPHENOL

D.1. METHODS

The multistage model included in U.S. EPA's BMD software (version 1.3.2) was fit to the censored incidence data for selected tumors in male and female $B6C3F_1$ mice exposed to either tPCP or commercial (EC-7) grade PCP in the diet for 2 years (NTP, 1989). The raw and censored incidence data are shown in Table D-1. Models were run restricting the fitted parameters to be positive, in order to fit a monotonically increasing dose-response relationship. The highest degree polynomial modeled for any data set was one less than the number of dose groups. For each data set, successive decreasing polynomial degrees (down to the one-degree) were modeled as well. Fit of a model to the data was assessed by the chi-square goodness-of-fit test. A χ^2 p-value $\geq$0.1 was considered to be an adequate fit (U.S. EPA, 2000b). Following U.S. EPA (2000b) methodology for the multistage model, the lowest degree polynomial that provided adequate fit was selected as the source of the risk estimate for that data set. As recommended in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a benchmark response (BMR) near the lower end of the observed data, generally a 10% increase in extra risk, was used.

Table D-1. Incidence of tumors in B6C3F$_1$ mice exposed to technical grade (tPCP) and commercial grade (EC-7) PCP in the diet for 2 years

Tumor type	tPCP (ppm in diet)			EC-7 (ppm in diet)			
	0	100	200	0	100	200	600
Males (mg/kg-day)[a]	0	18	35	0	18	37	118
Hepatocellular adenoma/carcinoma	7/32[b] (7/28)[d]	26/47[c] (26/46)	37/48[c] (37/46)	6/35[b] (6/33)	19/48[c] (19/45)	21/48[c] (21/38)	34/49[c] (34/47)
Adrenal benign/malignant pheochromocytoma	0/31[b] (0/26)	10/45[c] (10/41)	23/45[c] (23/44)	1/34[b] (1/32)	4/48 (4/45)	21/48[c] (21/39)	45/49[c] (45/47)
Females (mg/kg-day)[a]	0	17	35	0	17	34	114
Hepatocellular adenoma/carcinoma	3/33 (3/31)	9/49 (9/49)	9/50 (9/48)	1/34[b] (1/34)	4/50 (4/49)	6/49 (6/49)	31/48[c] (31/48)
Adrenal benign/malignant pheochromocytoma	2/33 (2/31)	2/48 (2/48)	1/49 (1/47)	0/35[b] (0/35)	2/49 (2/48)	2/46 (2/46)	38/49[c] (38/49)
Hemangioma/hemangio-sarcoma	0/35[b] (0/33)	3/50 (3/50)	6/50[c] (6/48)	0/35[b] (0/35)	1/50 (1/49)	3/50 (3/50)	9/49[c] (9/49)

[a]Average daily doses estimated by the researchers.
[b]Statistically significant trend ($p < 0.05$) by Cochran-Armitage test.
[c]Statistically significant difference from controls ($p < 0.05$) by Fisher Exact test.
[d]Censored data used for modeling are shown in parentheses; see text for description of censoring procedure.

Source: NTP (1989).

Although survival was considered by NTP (1989) to be adequate for evaluation of carcinogenicity in all groups, there were two survival-related issues that were considered for potential impact on the dose-response assessment. First, males in the control group for the tPCP study had unusually low survival, starting early in the study (first death at 15 weeks) and continuing to termination. Survival at termination was only 34%, compared with 71% in the EC-7 control males. The first hepatocellular tumor in this control group was observed in an animal that died at 48 weeks and the second in an animal that died at 60 weeks. Hepatocellular tumors in the low- and high-dose male tPCP groups were first observed at 59 and 54 weeks, respectively. These findings suggest that survival as short as 48 weeks was adequate for evaluation of liver tumors in the male mice. Despite the overall low survival and early onset of mortality in the male tPCP control group, there were still only five deaths that occurred in animals younger than 48 weeks. This compares to two deaths each in the low- and high-dose male tPCP groups in the same time frame. Therefore, survival issues in the control male tPCP group are expected to have little or no impact on the dose-response assessment.

The second survival-related issue was an increase in deaths occurring between weeks 40 and 80 in male mice in the mid-dose group in the EC-7 study (11 deaths, compared with 5 in controls, 7 in the low-dose group, and 4 in the high-dose group). Neither hepatocellular nor adrenal tumors were seen in any of these deaths among the mid-dose males. The earliest

appearance of these tumors in the male EC-7 study was 77 weeks for hepatocellular tumors and 66 weeks for adrenal pheochromocytomas, both in the high-dose group. However, as discussed above, hepatocellular tumors were seen as early as 48 weeks in untreated males in the tPCP study. Therefore, animals that died between 40 and 80 weeks in the EC-7 study were likely at risk of developing tumors, and the greater number of such animals in the mid-dose group versus the other groups is considered to be of little or no consequence for dose-response assessment.

Because survival issues were not expected to impact the dose-response assessment significantly, time-to-tumor modeling was not performed. However, as a standard adjustment to prevent counting animals that were never at risk of developing tumors, the incidence data were censored to remove animals that died before appearance in the experiment of the first tumor of the type in question in animals of the same sex and species (or 1 year, whichever occurred earlier).

Statistical analysis (Fisher Exact and χ^2 tests of 2 × 2 contingency tables) showed no difference in proportion of responders between male controls in the tPCP and EC-7 experiments for hepatocellular adenoma/carcinoma or adrenal benign/malignant pheochromocytoma, or between female controls in the tPCP and EC-7 experiments for hepatocellular adenoma/carcinoma, adrenal benign/malignant pheochromocytoma, or hemangioma/hemangiosarcoma. Therefore, dose-response analyses for each chemical formulation were conducted using the combined control groups.

In the NTP (1989) study, tumors were increased by PCP exposure at multiple sites–the liver and adrenal gland in both male and female mice. The females had increased circulatory tumors as well. There is a concern that in this situation a risk estimate based solely on one tumor type may underestimate the overall cancer risk associated with exposure to the chemical.

D.2. RESULTS

The BMD modeling results for the individual data sets are summarized in Table D-2. This table shows the BMDs and BMDLs derived from each endpoint modeled. BMDs and BMDLs presented in this table are those predicted by the multistage model selected according to U.S. EPA (2000b) BMD methods, at 10% extra risk. All data sets were run using combined control groups. Note that all risk estimates presented here are for mice; they have not been converted to human equivalent values.

Table D-2. Summary of BMD modeling results based on NTP (1989)

Endpoint	Test material	Model degree	Goodness of fit *p*-value	BMR, extra risk	BMD (mg/kg-day)	BMDL (mg/kg-day)
Males						
Hepatocellular adenoma/carcinoma	tPCP	one stage	0.597	10%	***2.84***	***2.15***
Adrenal pheochromocytoma/ malignant pheo	tPCP	one stage	0.382	10%	5.72	4.29
Hepatocellular adenoma/carcinoma	EC7	one stage	0.330	10%	10.6	7.62
Adrenal pheochromocytoma/ malignant pheo	EC7	two stage	0.159	10%	14.9	10.8
Females						
Hepatocellular adenoma/carcinoma	tPCP	one stage	0.336	10%	21.3	11.8
Hemangioma/ hemangiosarcoma	tPCP	one stage	0.998	10%	28.1	17.0
Hepatocellular adenoma/carcinoma	EC7	two stage	0.952	10%	37.7	22.9
Adrenal pheochromocytoma/ malignant pheo	EC7	Three stage	0.79	10%	47.7	34.6
Hemangioma/ hemangiosarcoma	EC7	one stage	0.986	10%	61.0	39.9

The appendix provides the detailed modeling results for each endpoint. The lowest BMD (2.84 mg/kg-day) and BMDL (2.15 mg/kg-day) were for hepatocellular adenomas/carcinomas in male mice treated with tPCP. BMDLs for other data sets ranged up to 20-fold higher. Dividing the extra risk level of 0.10 by the BMDL of 2.15 mg/kg-day yields an estimated slope factor of 0.046 $(mg/kg\text{-}day)^{-1}$ for PCP based on this endpoint (U.S. EPA, 2005a).

MODELING RESULTS BY ENDPOINT

Part 1. Hepatocellular adenoma/carcinoma in male B6C3F$_1$ mice treated with tPCP

adequate fit (p>0.1) with one-degree model

model fit details	χ^2	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	0.00	0	perfect fit	177.664	3.86	2.18
1 degree polynomial (pos betas)	**0.28**	**1**	**0.5970**	**175.945**	**2.84**	**2.15**

Combined controls
One-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 17:47:47 2006
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = tPCP_m_rp_l_cc
   Independent variable = tPCP_m_dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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


                  Default Initial Parameter Values
                     Background =     0.181278
                        Beta(1) =    0.0396975


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.57

   Beta(1)        -0.57            1


                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background            0.209317           0.109466
         Beta(1)           0.0371231         0.00901642
```

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-85.8322			
Fitted model	-85.9727	0.280935	1	0.5961
Reduced model	-106.048	40.4321	2	<.0001

AIC: 175.945

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.2093	12.768	13	61	0.023
i: 2					
18.0000	0.5947	27.355	26	46	-0.122
i: 3					
35.0000	0.7844	36.081	37	46	0.118

Chi-square = 0.28 DF = 1 P-value = 0.5970

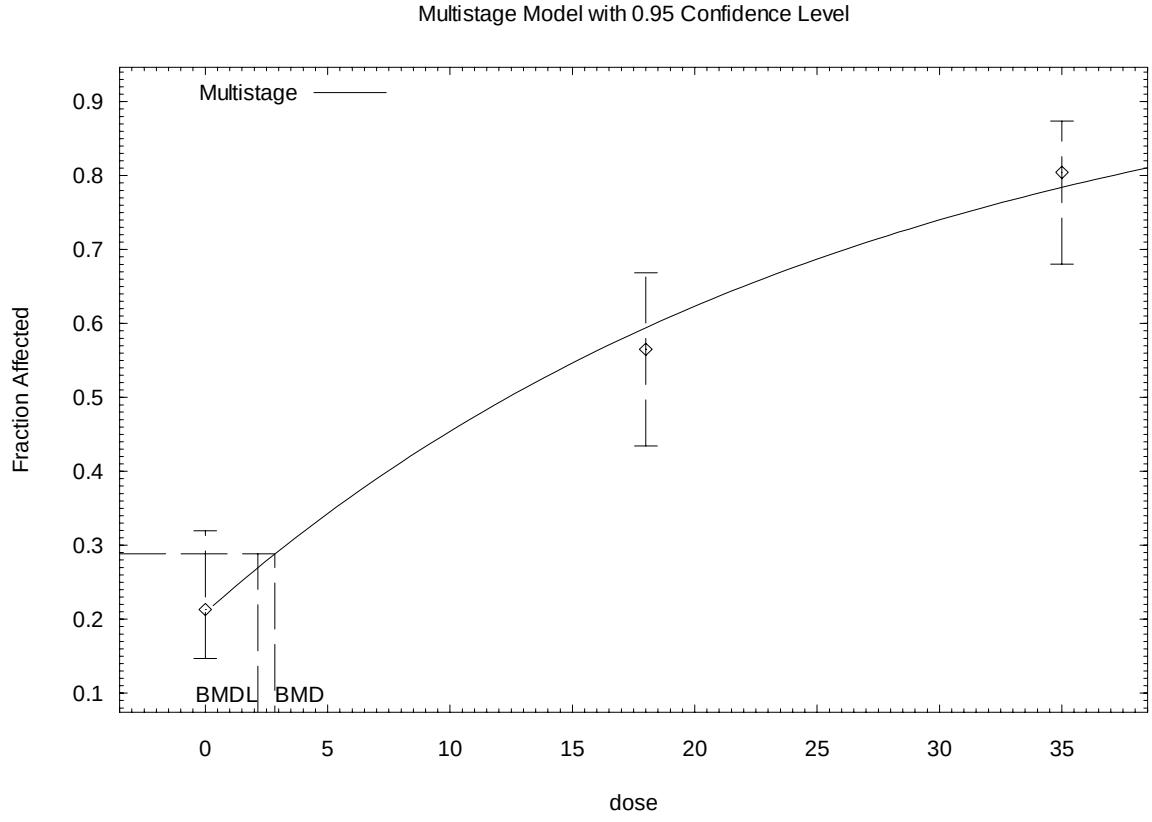
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.83814

BMDL = 2.15146



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Part 2. Adrenal pheochromocytoma/malignant pheochromocytoma in male B6C3F$_1$ mice treated with tPCP

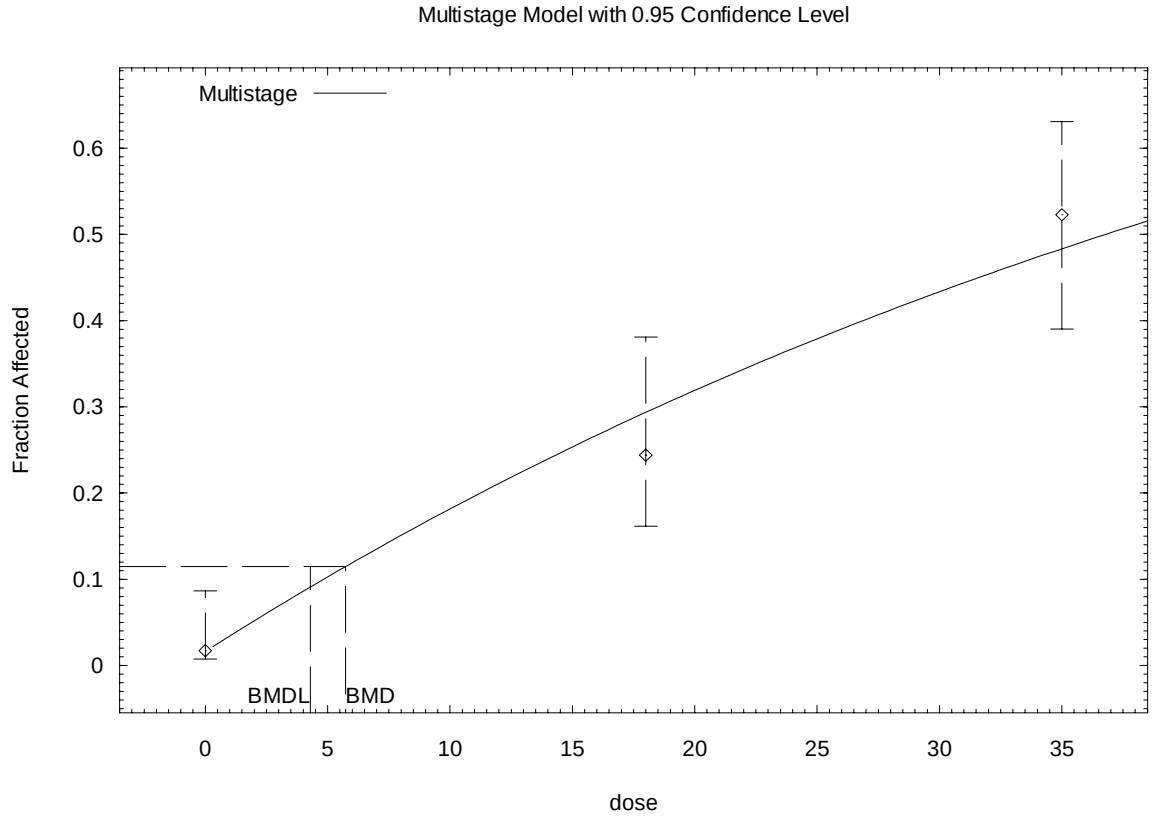
adequate fit (p>0.1) with one-degree model

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	0.00	0	perfect fit	122.564	9.22	4.48
1 degree polynomial (pos betas)	**0.77**	**1**	**0.3817**	**121.347**	**5.72**	**4.29**

Combined controls
One-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 17:50:33 2006
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = tPCP_m_rp_a_cc
   Independent variable = tPCP_m_dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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


                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =     0.020577


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.64

   Beta(1)        -0.64            1


                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background          0.0162929            0.121881
         Beta(1)          0.0184044          0.00665276


                        Analysis of Deviance Table

       Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model        -58.2818
```

```
    Fitted model        -58.6733      0.782979      1          0.3762
   Reduced model        -78.4336       40.3037      2         <.0001

            AIC:         121.347


                        Goodness  of  Fit

      Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ------------------------------------------------------------------------
i: 1
     0.0000     0.0163         0.945         1          58       0.059
i: 2
    18.0000     0.2937        12.041        10          41      -0.240
i: 3
    35.0000     0.4834        21.272        23          44       0.157

 Chi-square =       0.77     DF = 1        P-value = 0.3817

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        5.72473

            BMDL =        4.29098
```



Part 3. Hepatocellular adenoma/carcinoma in male B6C3F$_1$ mice treated with EC7

three- and two-degree models defaulted to the one-degree

adequate fit (p>0.1) with one-degree model

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
1 degree polynomial (pos betas)	**2.22**	**2**	**0.3298**	**238.389**	**10.61**	**7.62**

Combined controls
One-degree model

```
 ================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 17:52:55 2006
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = EC7_m_rp_l_cc
   Independent variable = EC7_m_dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


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



                  Default Initial Parameter Values
                     Background =     0.305226
                        Beta(1) =   0.00821465
                        Beta(2) =            0
                        Beta(3) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)    -Beta(3)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1         -0.6

   Beta(1)         -0.6            1


                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background           0.249937           0.0923968
         Beta(1)          0.00992673          0.00291281
         Beta(2)                   0                  NA
         Beta(3)                   0                  NA

NA - Indicates that this parameter has hit a bound
```
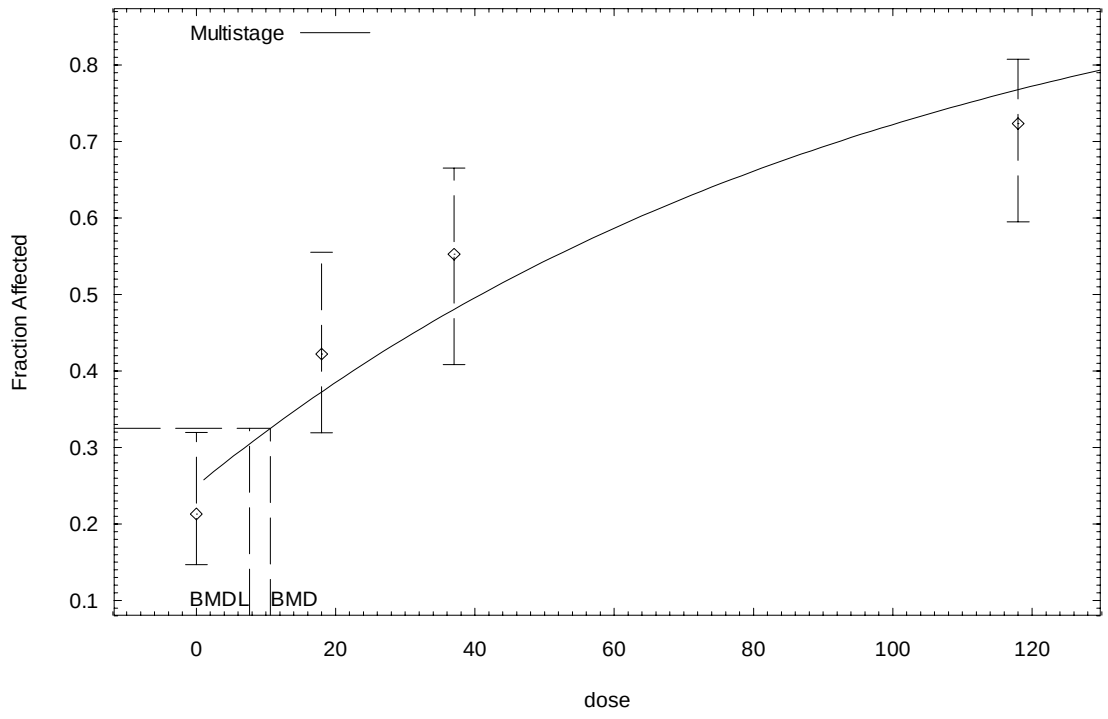

```
      implied by some inequality constraint and thus
      has no standard error.


                          Analysis of Deviance Table

        Model      Log(likelihood)  Deviance  Test DF     P-value
      Full model         -116.091
    Fitted model         -117.194       2.20623      2          0.3318
  Reduced model          -131.634       31.0845      3         <.0001

            AIC:          238.389


                       Goodness  of  Fit

      Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ------------------------------------------------------------------------
i: 1
    0.0000     0.2499        15.246        13          61      -0.196
i: 2
   18.0000     0.3727        16.770        19          45       0.212
i: 3
   37.0000     0.4805        18.259        21          38       0.289
i: 4
  118.0000     0.7675        36.073        34          47      -0.247

 Chi-square =       2.22     DF = 2        P-value = 0.3298


Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10.6138

            BMDL =        7.62123
```

Multistage Model with 0.95 Confidence Level



17:52 08/21 2006

Part 4. Adrenal pheochromocytoma/malignant pheochromocytoma in male B6C3F$_1$ mice treated with EC7

no adequate fit (p>0.1) with any models

model fit details	•^{2}	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	5.56	1	0.0184	119.263	12.50	7.25
1 degree polynomial (pos betas)	11.55	2	0.0031	125.816	5.75	4.61

High dose group dropped:

adequate fit (p>0.1) with two-degree model

model fit details	•^{2}	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	**1.98**	**1**	**0.1594**	**97.126**	**14.95**	**10.79**
1 degree polynomial (pos betas)	7.96	2	0.0048	103.899	7.81	5.63

High dose group dropped
Combined controls
Two-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 19:14:15 2006
 ======================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = EC7_m_rp_a_cc
   Independent variable = EC7_m_dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


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



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =           0
                        Beta(2) =  0.000576302
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(2)
Background	1	-0.53
Beta(2)	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0137997	0.107483
Beta(1)	0	NA
Beta(2)	0.00047164	0.000176465

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-45.4672			
Fitted model	-46.563	2.19157	1	0.1388
Reduced model	-67.6005	44.2666	2	<.0001

AIC: 97.126

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0138	0.800	1	58	0.253
i: 2					
18.0000	0.1536	6.910	4	45	-0.498
i: 3					
37.0000	0.4829	18.834	21	39	0.222

Chi-square = 1.98 DF = 1 P-value = 0.1594

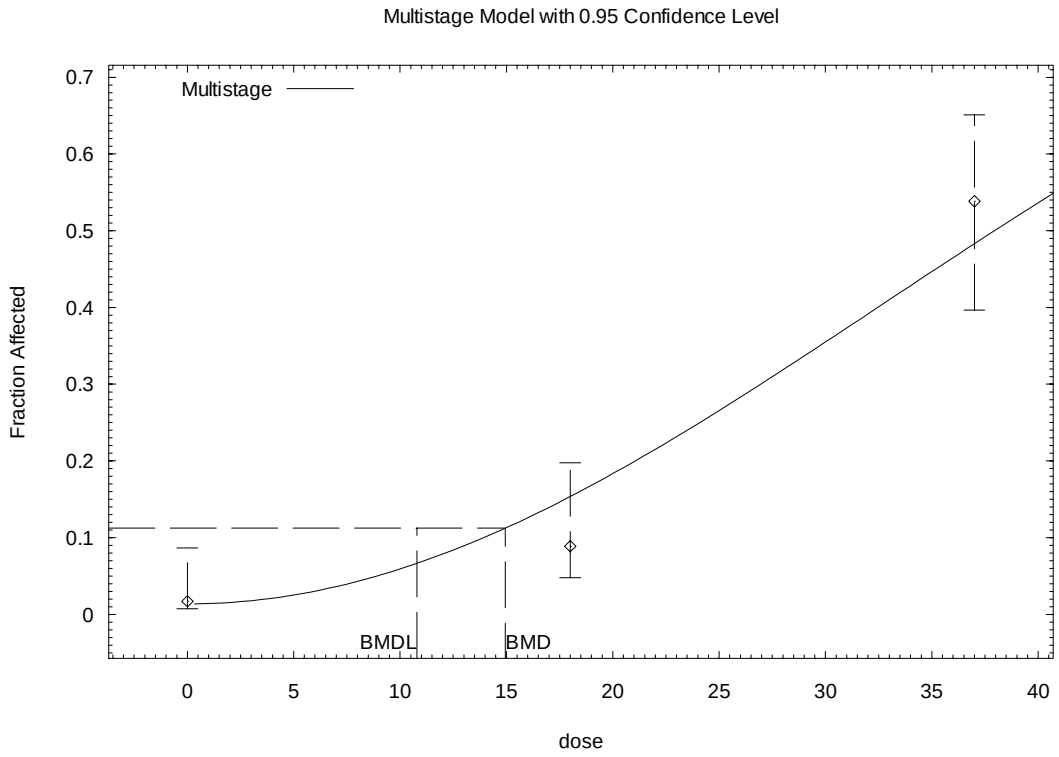
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 14.9463

BMDL = 10.7929



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Part 5. Hepatocellular adenoma/carcinoma in female B6C3F$_1$ mice treated with tPCP

two-degree model defaulted to the one-degree

adequate fit (p>0.1) with one-degree model

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
1 degree polynomial (pos betas)	**0.92**	**1**	**0.3362**	**128.013**	**21.27**	**11.79**

Combined controls
One-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 18:00:37 2006
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = tPCP_f_rp_l_cc
   Independent variable = tPCP_f_dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


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



                  Default Initial Parameter Values
                     Background =    0.0836063
                        Beta(1) =   0.00408011
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1        -0.74

   Beta(1)        -0.74            1



                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background           0.0688782           0.116196
         Beta(1)           0.0049533          0.00628285
         Beta(2)                   0                  NA

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-61.5594			
Fitted model	-62.0064	0.893896	1	0.3444
Reduced model	-64.3577	5.59665	2	0.06091

AIC: 128.013

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0689	4.477	4	65	-0.114
i: 2					
17.0000	0.1441	7.060	9	49	0.321
i: 3					
35.0000	0.2171	10.420	9	48	-0.174

Chi-square = 0.92 DF = 1 P-value = 0.3362

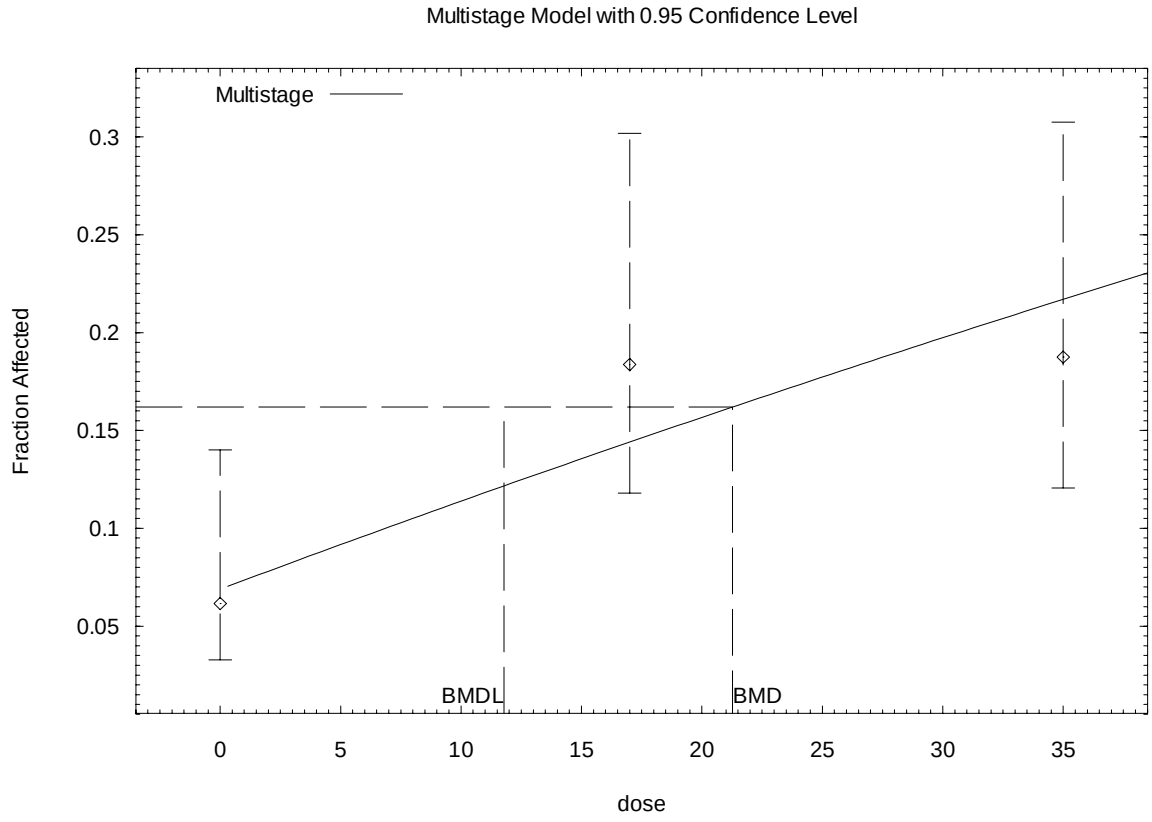
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 21.2708

BMDL = 11.7885



18:00 08/21 2006

Part 6. Hemangioma/hemangiosarcoma in female B6C3F$_1$ mice treated with tPCP

adequate fit (p>0.1) with one-degree model

model fit details	•^{2}	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	0.00	1	1.0000	62.8667	28.11	16.98
1 degree polynomial (pos betas)	**0.00**	**2**	**0.9978**	**60.8711**	**28.06**	**16.97**

Combined controls
One-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 18:10:12 2006
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = tPCP_f_rp_bl_cc
   Independent variable = tPCP_f_dose

 Total number of observations = 4
 Total number of records with missing values = 1
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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


                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =   0.00381681


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)            1


                           Parameter Estimates

        Variable           Estimate             Std. Err.
     Background                  0                 NA
        Beta(1)          0.00375481           0.0039077

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

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-29.4333			
Fitted model	-29.4356	0.00445262	2	0.9978
Reduced model	-34.9844	11.102	2	0.003884

AIC: 60.8711

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0000	0.000	0	68	0.000
i: 2					
17.0000	0.0618	3.092	3	50	-0.032
i: 3					
35.0000	0.1231	5.911	6	48	0.017

Chi-square = 0.00 DF = 2 P-value = 0.9978

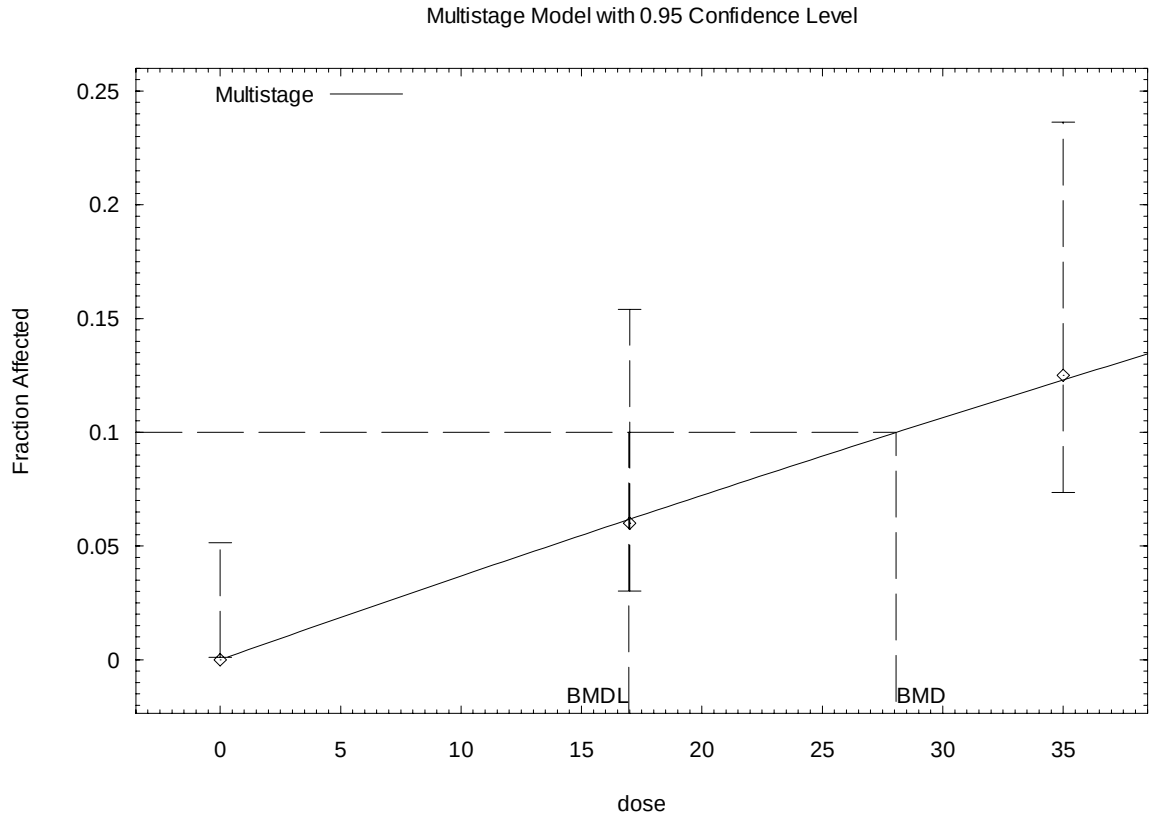
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 28.0602

BMDL = 16.972



18:10 08/21 2006

Part 7. Hepatocellular adenoma/carcinoma in female B6C3F$_1$ mice treated with EC7

adequate fit (p>0.1) with two-degree model

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	**0.10**	**2**	**0.9526**	**160.694**	**37.72**	**22.86**
1 degree polynomial (pos betas)	7.48	2	0.0238	168.686	16.51	12.48

Combined controls
Two-degree model

```
 ================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 18:14:37 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = EC7_f_rp_l_cc
   Independent variable = EC7_f_dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


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



                  Default Initial Parameter Values
                     Background =    0.0555416
                        Beta(1) =            0
                        Beta(2) = 7.53898e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(2)

Background            1         -0.4

   Beta(2)         -0.4            1


                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background             0.05897           0.0797484
         Beta(1)                   0                  NA
         Beta(2)         7.4039e-005        1.99625e-005

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
```

has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-78.2973			
Fitted model	-78.347	0.0992897	2	0.9516
Reduced model	-109.352	62.1099	3	<.0001

AIC: 160.694

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0590	3.833	4	65	0.046
i: 2					
17.0000	0.0789	3.866	4	49	0.038
i: 3					
34.0000	0.1362	6.672	6	49	-0.117
i: 4					
114.0000	0.6405	30.743	31	48	0.023

Chi-square = 0.10 DF = 2 P-value = 0.9526

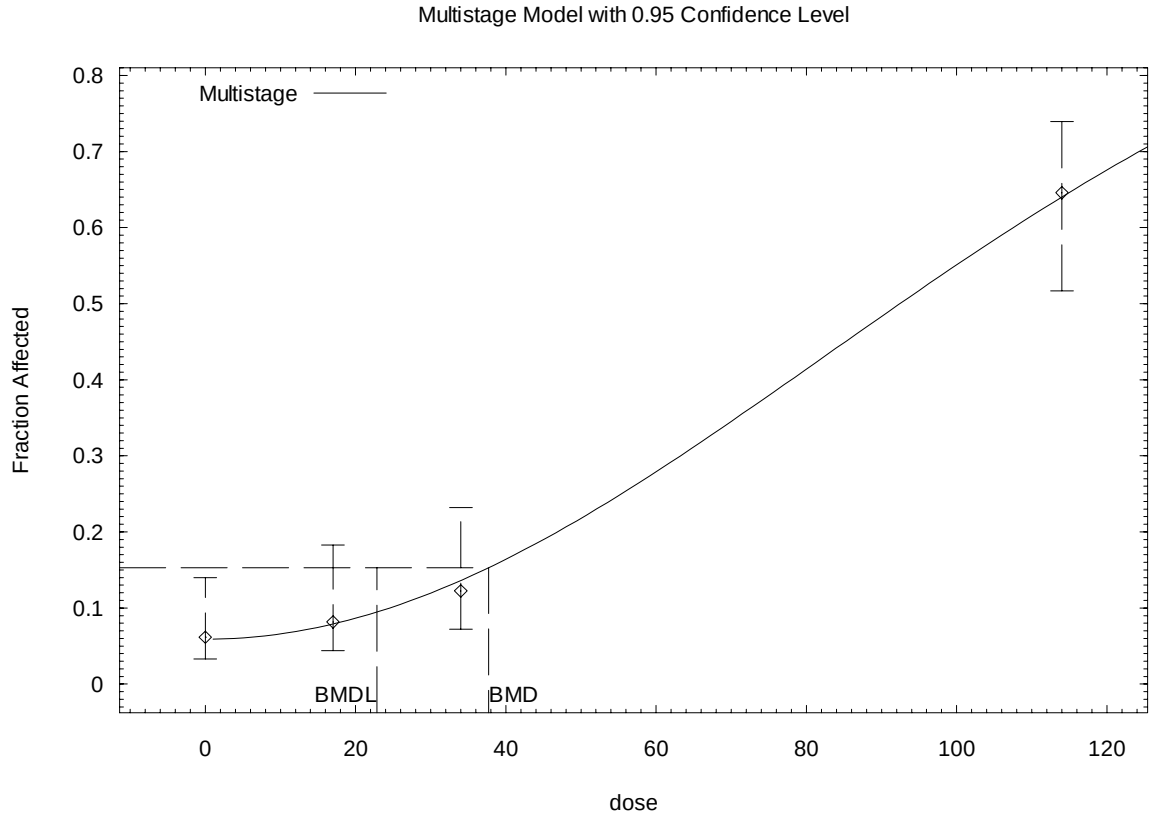
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 37.7232

BMDL = 22.8618



18:14 08/21 2006

Part 8. Adrenal pheochromocytoma/malignant pheochromocytoma in female B6C3F$_1$ mice treated with EC7

Adequate fit (p>0.1) with ≥ two-degree models, no adequate fit with one-degree model.

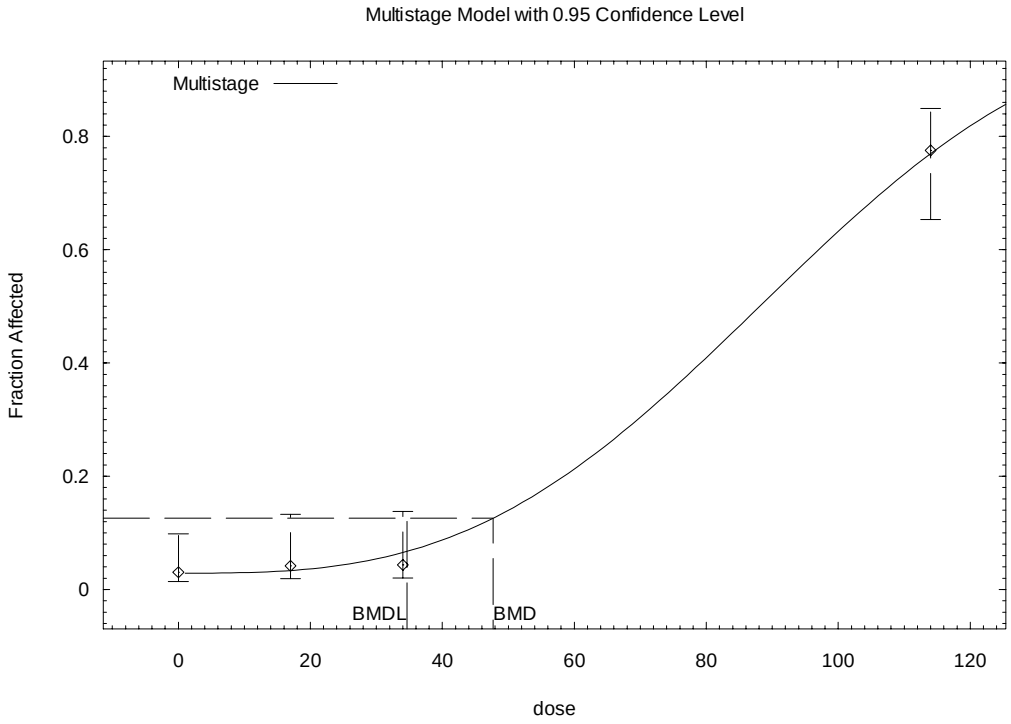
Three-stage model, with only the third stage coefficient fit, had the lowest AIC.

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
4 degree polynomial (pos betas)	0.08	1	0.7711	109.277	58.05	35.88
3 degree polynomial (pos betas)	**0.47**	**2**	**0.7903**	**107.703**	**47.69**	**34.65**
2 degree polynomial (pos betas)	3.75	2	0.1537	111.771	32.44	26.92
1 degree polynomial (pos betas)	21.43	2	0.0000	133.837	13.99	10.81

Combined controls
Three-degree model

```
 ================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                Mon Aug 21 18:35:44 2006
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = EC7_f_rp_a_cc
   Independent variable = EC7_f_dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


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



                  Default Initial Parameter Values
                     Background =    0.0245017
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 9.91296e-007


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by the user,
                 and do not appear in the correlation matrix )

             Background      Beta(3)

Background            1        -0.28
```

```
   Beta(3)        -0.28            1


                          Parameter Estimates

        Variable           Estimate            Std. Err.
      Background            0.028872           0.0787936
         Beta(1)                   0                  NA
         Beta(2)                   0                  NA
         Beta(3)        9.71404e-007        2.08593e-007

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


                         Analysis of Deviance Table

        Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -51.5972
   Fitted model         -51.8514      0.508423      2          0.7755
  Reduced model         -107.563       111.931      3         <.0001

            AIC:          107.703


                       Goodness  of  Fit

      Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
  ------------------------------------------------------------------------
i: 1
    0.0000     0.0289         1.906         2          66       0.051
i: 2
   17.0000     0.0335         1.608         2          48       0.252
i: 3
   34.0000     0.0653         3.002         2          46      -0.357
i: 4
  114.0000     0.7697        37.716        38          49       0.033

 Chi-square =       0.47     DF = 2        P-value = 0.7903


Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        47.6898

             BMDL =        34.6479
```



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Part 9. Hemangioma/hemangiosarcoma in female B6C3F$_1$ mice treated with EC7

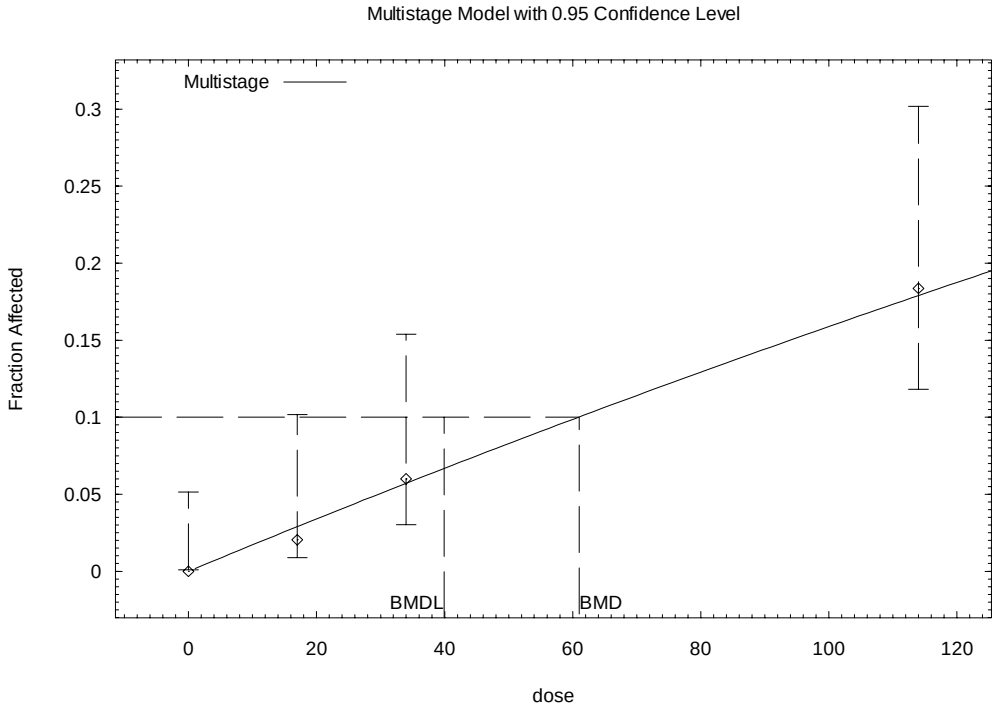
adequate fit (p>0.1) with models of all degrees, so choose simplest (one-degree)

model fit details	$\bullet^2$	df	p-value for model fit	AIC for fitted model	BMD (mg/kg)	BMDL (mg/kg)
2 degree polynomial (pos betas)	0.11	2	0.9449	83.3146	63.01	40.03
1 degree polynomial (pos betas)	**0.14**	**3**	**0.9862**	**81.3551**	**61.02**	**39.91**

Combined controls
One-degree model

```
 ======================================================================
         Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
         Input Data File: C:\BMDS\DATA\PCP-REV.(d)
         Gnuplot Plotting File:  C:\BMDS\DATA\PCP-REV.plt
                                                  Mon Aug 21 18:39:55 2006
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = EC7_f_rp_bl_cc
   Independent variable = EC7_f_dose

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


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



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =   0.00180953


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)           1


                           Parameter Estimates

        Variable           Estimate            Std. Err.
      Background                  0                 NA
         Beta(1)          0.00172662          0.00128595
```

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


                        Analysis of Deviance Table

        Model      Log(likelihood)  Deviance  Test DF     P-value
     Full model         -39.5989
    Fitted model        -39.6775      0.157225      3          0.9842
   Reduced model         -49.135       19.0721      3       0.0002642

            AIC:         81.3551
                      Goodness  of  Fit

      Dose     Est._Prob.    Expected    Observed     Size     Chi^2 Res.
   ------------------------------------------------------------------------
i: 1
     0.0000     0.0000         0.000         0          68       0.000
i: 2
    17.0000     0.0289         1.417         1          49      -0.303
i: 3
    34.0000     0.0570         2.851         3          50       0.056
i: 4
   114.0000     0.1787         8.755         9          49       0.034

 Chi-square =       0.14     DF = 3        P-value = 0.9862


Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        61.0211

            BMDL =        39.9095
```



APPENDIX E: COMBINED ESTIMATES OF CARCINOGENIC RISK

Considering the multiple tumor types and sites observed in the mice exposed to PCP, the estimation of risk based on only one tumor type/site may underestimate the overall carcinogenic potential of PCP. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) identify two ways to approach this issue—analyzing the incidences of tumor-bearing animals, or combining the potencies associated with significantly elevated tumors at each site. The NRC (1994) concluded that an approach based on counts of animals with one or more tumors would tend to underestimate overall risk when tumor types occur independently, and that an approach based on combining the risk estimates from each separate tumor type should be used.

Because potencies are typically upper bound estimates, combining such upper bound estimates across tumor sites is likely to overstate the overall risk. Therefore, following the recommendations of the NRC (1994) and the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), a statistically valid upper bound on combined risk was derived in order to gain some understanding of the overall risk resulting from tumors occurring at multiple sites. It is important to note that this estimate of overall potency describes the risk of developing tumors at any combination of the sites considered, and is not just the risk of developing tumors at all three sites simultaneously.

For individual tumor data modeled using the multistage model,

$$(1)\ P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

the model for the combined tumor risk is still multistage, with a functional form that has the sum of stage-specific multistage coefficients as the corresponding multistage coefficient;

$$(2)\ P_c(d) = 1 - \exp[-(\Sigma q_{0i} + d\Sigma q_{1i} + d^2\Sigma q_{2i} + \ldots + d^k\Sigma q_{ki})],$$ for i = 1,…, m (m = total number of sites).

The resulting equation for fixed extra risk (BMR) is polynomial in dose (when logarithms of both sides are taken) and can be straightforwardly solved for combined BMD. But confidence bounds for that BMD are not estimated by available benchmark dose software (e.g., BMDS).

The NRC (1994) also recommended an approach based on simulations. Therefore, a bootstrap analysis (Efron and Tibshirani, 1993) was used to derive the distribution of the BMD for the combined risk of liver and adrenal gland tumors observed in male rats with oral exposure to PCP. Within each of the individual tumor data sets (see Table E-1), a simulated incidence level was generated for each exposure group using a binomial distribution with probability of

success estimated by a Bayesian (assuming a flat prior) estimate of probability given by (observed incidence+1)/(total number+2). This adjustment is necessary in order to avoid underestimation of variability when the observed incidence is 0 in any group, and then must be applied to all groups to preserve the differences between them. Then each simulated data set was modeled using the multistage model in the same manner as those reported in Appendix D above. The multistage parameter estimates from the individual tumors were substituted in the equation (2) above, which was solved for the BMD at an overall benchmark response of 1% extra risk. This process was repeated until there were 10,000 simulated experiments for each individual tumor. Whenever the multistage model could not provide an adequate fit for any of the simulated data sets, the simulated experiments were excluded from the analysis. Then the 5^{th} percentile from the distribution of combined BMDs was used to estimate the lower 95% bound on the dose (BMDL) corresponding to an extra risk of 1% for any of the three tumor sites.

The results of combining risks across sites within data sets are shown in Table 5-6. The highest combined risk observed, similarly to the individual cancer risk estimates, was in tPCP-exposed male mice. The 95% UCL on the combined risk for animals that developed liver and/or adrenal gland tumors was 4.0×10^{-1} $(mg/kg\text{-}day)^{-1}$, which is about 30% higher than the 3.1×10^{-1} $(mg/kg\text{-}day)^{-1}$ cancer slope factor estimated from liver tumors only in tPCP-exposed male mice. The risk estimates for the tPCP-exposed males and females tend to be higher than those for the EC-7-exposed animals, by approximately twofold for the central tendency estimates and for the upper bound estimates.

Table E-1. Results of simulation analyses characterizing combined cancer risk estimates for male and female mice (NTP, 1989)

Endpoint	In terms of administered bioassay exposures		Human equivalents[a]			
	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	$BMD_{10/HED}$ mg/kg-day	$0.1/BMD_{10/HED}$ $(mg/kg\text{-}day)^{-1}$	$BMDL_{10.HED}$ mg/kg-day	$0.1/BMDL_{10/HED}$ $(mg/kg\text{-}day)^{-1}$
Male mice, tPCP						
Hepatocellular adenoma/carcinoma	3.12	2.27	0.475	0.211	0.35	0.290
Adrenal benign/malignant pheochromocytoma	6.45	4.47	0.981	0.102	0.68	0.147
Combined tumors	2.23	1.63	0.340	0.294	0.25	0.402
Male mice, EC-7						
Hepatocellular adenoma/carcinoma	11.0	7.59	1.68	0.060	1.15	0.087
Adrenal benign/malignant pheochromocytoma	12.6	5.75	1.92	0.052	0.88	0.114
Combined tumors	6.2	3.7	0.944	0.106	0.57	0.174
Female mice, tPCP						
Hepatocellular adenoma/carcinoma	21.3	11.7	3.24	0.031	1.79	0.056
Hemangioma /hemangiosarcoma	27.8	16.3	4.23	0.024	2.48	0.040
Combined tumors	12.6	7.88	1.91	0.052	1.20	0.083
Female mice, EC-7						
Hepatocellular adenoma/carcinoma	36.9	16.4	5.61	0.018	2.50	0.040
Adrenal benign/malignant pheochromocytoma	45.5	29.6	6.93	0.014	4.51	0.022
Hemangioma /hemangiosarcoma	60.7	37.9	9.24	0.011	5.76	0.017
Combined tumors	23.2	13.6	3.52	0.028	2.07	0.048

[a]HED (mg/kg-day) = dose in animals (mg/kg-day) × $(BW_a/BW_h)^{0.25}$

At 0.037 kg for male mice and 0.038 kg for female mice and 70 kg for humans, the cross-species scaling factor was 0.15.