OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

1,4-Dichlorobenzene

Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Public Review Draft

November 2024

Air and Site Assessment and Climate Indicators Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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Appendix D1

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List of Abbreviations

AIC	Akaike Information Criterion
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry, The
BMC	Benchmark concentration
$BMCL_{05}$	The 95% lower confidence limit of the dose producing a 5% response rate
BMD	Benchmark dose
BMDL	Lower confidence limit of the benchmark dose
BMI	Body mass index
BMR	Benchmark response
BUN	Blood urea nitrogen
BW	Body weight
CARB	California Air Resources Board, The
CBQ	Chlorobenzoquinone
CDC	US Centers for Disease Control and Prevention, The
CNS	Central nervous system
CPN	Chronic progressive nephropathy
Cr	Creatinine
CVD	Cardiovascular disease
CYP450	Cytochrome P450
DCBQ	Dichlorobenzoquinone
DCC	Dichlorocatechol
DCGHQ	Dichlorogluthionylhydroquinone
DCHQ	Dichlorohydroquinone
°C	Degrees Celsius
DPR	California Department of Pesticide Regulation, The
FEV_1	Forced expiratory volume
F_0	Parental generation
F_1	First offspring generation
F_2	Second filial generation
GD	Gestation day
GM	Geometric mean
GSH	Glutathione
g/cm^3	Grams per cubic centimeter
g/mol	Grams per mole
HEC	Human Equivalent Concentration
lbs	Pounds
LDH	Lactose dehydrogenase
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of detection
mg/m^3	Milligrams per cubic meter
mg/g	Milligrams per gram
mg/kg-day	Milligrams per kilogram per day
mg/L	Milligrams per liter
mm Hg	Millimeters mercury
$\mu g/m^3$	Micrograms per cubic meter
MMEFR	Maximum mid-expiratory flow rate
MRL	Minimal Risk Level (ATSDR)
NHANES	National Health and Nutrition Examination Survey, The
NOAEL	No Observed Adverse Effect Level
NSRL	No Significant Risk Level
NTP	National Toxicology Program
NZW	New Zealand White (rabbits)
OEHHA	Office of Environmental Health Hazard Assessment, The
1,4-DCB	1,4-Dichlorobenzene

List of Abbreviations (continued)

PBPK	Physiologically-based pharmacokinetic
PND	Postnatal day
POD	Point of departure
ppb	Parts per billion
ppm	Parts per million
RBC	Red blood cell
REL	Reference Exposure Level
RfC	Reference Concentration
RGDR	Regional gas dose ratio
SG	Glutathione-S-yl-metabolite
SRP	Scientific Review Panel
TAC	Toxic Air Contaminant
TSD	Technical Support Document
TWA	Time-weighted average
2,5-DCP	2,5-Dichlorophenol
2,4-DCP	2,4-Dichlorophenol
UF	Uncertainty factor
UF_{A-d}	Interspecies Toxicodynamic Uncertainty Factor
UF_{A-k}	Interspecies Toxicokinetic Uncertainty Factor
UF_{H-d}	Intraspecies Toxicodynamic Uncertainty Factor
UF_{H-k}	Intraspecies Toxicokinetic Uncertainty Factor
UF_L	LOAEL Uncertainty Factor
UF_S	Subchronic Uncertainty Factor
US EPA	United States Environmental Protection Agency, The
VOC	Volatile organic compound
WBC	White blood cell

Preface

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). Pursuant to this mandate, OEHHA developed a Technical Support Document (TSD), adopted in 2008, that describes methodologies for deriving acute, 8-hour and chronic Reference Exposure Levels (RELs).

RELs are airborne concentrations of a chemical that are not anticipated to result in adverse noncancer health effects for specified durations in the general population and sensitive subpopulations. In particular, the methodology explicitly considers possible differential effects on the health of infants, children, and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 et seq.).

The acute, 8-hour, and chronic RELs for 1,4-dichlorobenzene in this document were developed using the process described above. RELs are completed using the public process outlined in HSC section 44360(b)(2). This process includes public comment and review by the Scientific Review Panel (SRP) on Toxic Air Contaminants. When finalized, the RELs are adopted into Appendix D of the TSD.

This document is being released for public comment via written submissions and public workshops in Northern and Southern California. Because of the scientific information contained in this document, additional explanations of concepts and terms are provided. These explanations appear in the main text and sometimes in footnotes. Therefore, those using reading-assistive software should enable the pronunciation of punctuation and symbols and listen for links to footnoted text. OEHHA's website has information about how to engage in the public review process. The comment period closes on January 13, 2025. Public comments will be considered in the revised draft document, which will be reviewed by the SRP.

1,4-Dichlorobenzene Reference Exposure Levels

(p-dichlorobenzene; para-dichlorobenzene; di-chloricide; p-chlorophenyl chloride)

CAS: 106-46-7

1. Summary

1.1 1,4-Dichlorobenzene Acute REL

Reference Exposure Level	**8,700 micrograms per cubic meter (µg/m^3; 1500 parts per billion (ppb))**
Critical effect(s)	Decreased birth weight and viability in newborn rat pups; blood vessel anomaly (retroesophageal right subclavian artery) in fetal rabbits
Hazard index target(s)	Development

1.2 1,4-Dichlorobenzene Chronic REL

Reference Exposure Level	**5.0 µg/m^3 (0.8 ppb)**
Critical effect(s)	Degenerative changes to nasal olfactory epithelium in female rats; mineralization of the testis in male mice
Hazard index target(s)	Respiratory system male reproductive system

1.3 1,4-Dichlorobenzene 8-Hour REL

Reference Exposure Level	**10 µg/m^3 (1.7 ppb)**
Critical effect(s)	Degenerative changes to nasal olfactory epithelium in female rats; mineralization of the testis in male mice
Hazard index target(s)	Respiratory system, male reproductive system

Acute: Acute exposure to 1,4-dichlorobenzene (1,4-DCB) has been found to cause nasal and eye irritation following acute occupational exposure in humans. Biomonitoring surveys in pregnant women observed associations between increased

levels of a 1,4-DCB urinary metabolite (2,5-dichlorophenol) and low infant birth weights as well as increased odds for respiratory and allergic outcomes. In a two-generation study, gestational 1,4-DCB exposure in female rats resulted in decreased viability and low birth weight in newborn pups. The developmental effects in newborn pups in the two-generation study is the basis for the Acute REL.

Chronic and 8-hour: Human case studies of repeated intentional 1,4-DCB exposure (e.g., substance abuse) by either inhalation or ingestion show central nervous system toxicity and brain damage. Biomonitoring surveys of human populations observed associations between earlier puberty onset in girls and higher urinary 2,5-dichlorophenol levels, suggesting that 1,4-DCB may alter hormonal activity in children. Controlled chronic inhalation exposure of rodents to 1,4-DCB resulted in nasal olfactory epithelium degeneration in female rats, and testis mineralization in male mice. Benchmark dose modeling of nasal lesions in female rats were used to derive the Chronic and 8-hr RELs.

Background: The Chronic REL presented in this document will supersede the previous Chronic REL of 800 µg/m^3 adopted for 1,4-DCB in 2000. A Cancer Inhalation Unit Risk Factor of 1.1×10^{-5} per microgram per cubic meter (µg/m^3)$^{-1}$ for 1,4-DCB is listed in the Air Toxics Hot Spots Program Table of Unit Risk and Cancer Potency Values (OEHHA, 2023). 1,4-DCB is also on the California Proposition 65 list as a chemical known to cause cancer and has a No Significant Risk Level (NSRL) of 20 µg/day for drinking water (OEHHA, 2022).

Literature Review: This document contains relevant published material, and relevant unpublished studies reviewed and supported by authoritative bodies. An extensive literature search was conducted to identify human or animal studies on the toxic effects of 1,4-DCB. The initial search was conducted in May 2020 and was updated periodically through July 2024. Searches were executed in PubMed, Embase, Scopus and SciFinder. Synonyms for 1,4-DCB were identified using USAPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/), and PubMed's MeSH database (https://www.ncbi.nlm.nih.gov/mesh/). The search was run initially in PubMed, then the search terms and syntax were adapted to suit the other databases used. In addition to the formal database searches, the reference lists of included papers and later publications that cited included papers were reviewed and periodic keyword searches were done in internet search engines, such as Google Scholar. A technical review of those studies specifically applicable to developing noncancer acute, 8-hour, and chronic inhalation RELs for 1-4-DCB is included.

2. Physical & Chemical Properties

Source: PubChem (2020), unless noted otherwise

Description	Colorless or white crystalline solid that sublimes at ambient temperature
Molecular formula	$C_6H_4Cl_2$
Molecular weight	147.01 grams per mole (g/mol)
Conversion factor	1 ppm = 6.01 milligrams per cubic meter (mg/m^3) @ 25 °C
Density	1.2475 grams per cubic centimeter (g/cm^3) @ 20 °C
Boiling point	174 °C
Melting point	52.7 °C
Vapor pressure	1.74 millimeters of mercury (mm Hg) @ 25 °C,
Odor threshold in air	1.1 mg/m^3 [0.2 parts per million (ppm)]; Amoore and Hautala, 1983)
Odor characteristics	Has a penetrating, distinctive aromatic or mothball-like odor that becomes very strong at concentrations above 30 to 60 ppm (180 to 360 mg/m^3)
Solubility	Soluble in benzene, ethanol, ether, acetone, and carbon disulfide. Practically insoluble in water (81.3 milligrams per liter (mg/L) at 25 °C).
Log octanol/water partition coefficient	3.44

3. Major Uses, Occurrence and Exposures

1,4-DCB is an organic chlorinated compound used as a deodorant for toilets, urinals, and refuse containers; as a moth repellant to protect clothing; and as a fumigant to control mold (PubChem, 2020). Consequently, the indoor air in homes and workplaces are the most common locations of exposure, although measurable levels of 1,4-DCB are also found in outdoor urban environments (Wallace, 1986; Yoshida et al., 1998; Yoshida et al., 2021).

1,4-DCB is also used in the manufacture of polyphenylene sulfide by reaction with a suitable sulfur source, such as sodium sulfide (ATSDR, 2006). Polyphenylene sulfide is an engineering thermoplastic that is widely used in the electronics, automotive,

aerospace, and chemical industries. Additional uses as an intermediate occur in the manufacture of other plastics and resins, pesticides, fertilizers, and synthetic dyes and pigments (EPA, 2020). 1,4-DCB has some limited uses in commercial and consumer products, including use in degreasers in oil additives for engines and pneumatic tools, use as a fuel additive for gasoline and diesel, and use in foam insulation and foam sealant in building and construction products.

1,4-DCB has been identified as a Hazardous Air Pollutant pursuant to subsection (b) of Section 112 of the federal Clean Air Act (42 U.S.C. Section 7412(b)) and was designated by the California Air Resources Board (CARB) to be a Toxic Air Contaminant pursuant to Health and Safety Code Section 39657 (CARB, 1993). To reduce both indoor and near-source outdoor air concentrations of 1,4-DCB, CARB implemented a ban on the sale and manufacture of solid air fresheners or toilet/urinal care products that contain 1,4-DCB, effective December 31, 2006 (CARB, 2004). However, 1,4-DCB continues to be used in mothballs in California, and is also registered by the California Department of Pesticide Regulation (DPR) for use as a pesticide in residential and commercial spaces (DPR, 2021). A total of 491,453 pounds (lbs) of 1,4-DCB in pesticide products was sold in California in 2018. This total did not include all residential uses, since reporting of residential pesticide use is not required in California.

California stationary source facilities that reported the highest emissions of 1,4-DCB (between 100 and 2100 lbs/year) in 2020 under the Hot Spots Program included sawmills/lumber producers, wastewater management and water treatment facilities, landfills, biomass power plants, and cheese making facilities (CARB, 2022a).

Between 1990 and 2007, CARB included 1,4-DCB in their California statewide outdoor ambient air monitoring of numerous toxic substances (CARB, 2022b). Air monitoring routinely took place at about 20 urban sites throughout the year. For 1,4-DCB, the number of observations per year ranged from 124 to 626. In most air samples, the air concentration of 1,4-DCB was below the limit of detection (LOD) of 0.2 to 0.3 parts per billion (ppb). Maximum levels ranged from 0.4 to 3.1 ppb between 1990 and 2005 and may represent emissions near facilities that use 1,4-DCB.

A study conducted in 1987 by CARB in Los Angeles County was undertaken to determine the personal, indoor, and outdoor exposure concentrations of 25 volatile organic compounds (VOCs), including 1,4-DCB (Wallace et al., 1991). Fifty-one homes were tested in February of 1987, and 43 were revisited in July 1987 to study the seasonal differences of the VOCs. For household characteristics and activities, the percentage of those that had ever used mothballs, indoor air fresheners, and bathroom deodorants (products that may contain 1,4-DCB) was 2%, 71%, and 22% of households, respectively. 1,4-DCB was measurable in 59% of the initial breath

samples and 77% of the personal air samples [LOD was 0.05 to 0.15 micrograms per cubic meter (µg/m^3)]. The mean residential indoor concentration of 1,4-DCB was 37 µg/m^3 (6.1 ppb) with a maximum of 330 µg/m^3 (55 ppb). Outdoor air levels of 1,4-DCB in the backyards of the homes were lower, ranging between 1 and 2 µg/m^3 (0.17–0.33 ppb). Arithmetic mean residential indoor air concentrations of 1,4-DCB were higher in living areas in the winter (27 µg/m^3) than in the summer (7.2 µg/m^3). The ratios of arithmetic mean indoor air concentrations to outdoor air concentrations for 1,4-DCB (ratio = 15 in winter, ratio = 12 for summer) were among the highest of the VOCs investigated, indicating a strong tendency for indoor use and exposure.

Twenty-one VOCs, including 1,4-DCB, were measured in 2000–2001 in 20 classrooms of 7 different Los Angeles area schools (13 portables and 7 main building rooms) during the cooling and heating season (Shendell et al., 2004). Passive clip-on monitors set up on top of a shelf or cabinet were used to measure the VOCs in classrooms during school hours. The concentration of 1,4-DCB was generally very low, ranging from not detectable to 10.6 µg/m^3 (1.8 ppb) with a mean level of 2.6 µg/m^3 (0.43 ppb).

4. Toxicokinetics

Based on the volatility of 1,4-DCB, inhalation is the most likely route for human exposure (ATSDR, 2006). 1.4-DCB is not appreciably absorbed through intact skin. Significant oral exposure is likely to be limited to accidental or incidental ingestion.

Inhalation studies in rats show that the highest tissue peak concentration of 1,4-DCB occurs in fat, with lower peak concentrations in liver, kidney, and serum. 1,4-DCB concentrations in these tissues decline to low levels 24 hours following exposure cessation, indicating that storage of 1,4-DCB in fat is not long-term. 1,4-DCB is primarily metabolized in the liver via cytochrome P450 (CYP450) to an epoxide, followed by further oxidation to 2,5-dichlorophenol (2,5-DCP), with minor amounts of 2,4-dichlorophenol (2,4-DCP). The dichlorophenols are primarily eliminated in urine following secondary metabolism. In humans, dichlorophenol conjugation with glutathione (GSH) appears to be the major metabolite found in urine, with smaller amounts of glucuronide and sulfate conjugates. Considerably lesser amounts of the metabolites are eliminated in feces and exhaled breath. 1,4-DCB and its metabolites decline to very low levels in these matrices 72 hours after exposure cessation.

4.1 Toxicokinetic Studies in Animals

The kinetics of radiolabeled 1,4-DCB has been studied via oral, subcutaneous, and inhalation administration in CFY female rats, a Sprague-Dawley derived strain (Hawkins et al., 1980). After single [50–500 milligrams per kilogram (mg/kg)] or multiple [250 mg/kg per day (mg/kg-day) for 10 days] oral exposures of radiolabeled

1,4-DCB in rats, radioactivity was detected in the liver, kidneys, lungs, muscle, fat, and blood plasma, indicating that considerable absorption had occurred. In addition, data showed that levels in tissues were similar following 10 oral exposures or 10 subcutaneous injections of 250 mg/kg, indicating almost complete absorption. The radiolabel levels in tissues did not appreciably increase with an increasing number of exposures beyond one, indicating a lack of bioaccumulation.

Twenty-four hours after cessation of inhalation exposure to 1000 parts per million (ppm; 6000 mg/m^3) for 3 hours/day, for 10 days, Hawkins et al. (1980) found that the 1,4-DCB lung concentrations were not as high as those found after exposure via other routes of administration. This finding indicated that 1,4-DCB was rapidly absorbed and cleared from the lungs following inhalation exposure. Following oral (250 mg/kg-day oral gavage in sunflower oil for 10 days) or inhalation (1000 ppm, 3 hours/day for 10 days) exposure in rats, elimination was primarily urinary, with 91%–97% of the total recovered label found in the urine by day 5 post-exposure. Elimination in the expired air was negligible, at 1% or less of the total excreted label.

In the same study, tissue distribution of radiolabeled 1,4-DCB was studied in female CFY rats after repeated administration via inhalation (1000 ppm), or subcutaneous or oral doses (250 mg/kg-day; Hawkins et al., 1980). After 24 hours, the kinetics of tissue distribution was similar between all routes of exposure. The highest level of radioactivity was found in kidneys, fat, liver, and lungs. Comparisons of 1,4-DCB tissue concentrations during repeated exposures showed that concentrations were lower after 10 daily exposures than after 6 daily exposures, possibly due to induction of metabolism.

In a pharmacokinetic study with Fischer 344 (F344) male and female rats and male and female B6C3F$_1$ mice, the absorption of 1,4-dichloro[^{14}C]benzene (^{14}C-1,4-DCB) by the oral and inhalation routes was investigated (Wilson et al., 1990). In rats, oral exposures were conducted at a single dose of 149 or 305 mg/kg/day and a repeated oral dose of 309 mg/kg/day. Inhalation exposures were conducted in male rats at 160 or 502 ppm (962 or 3017 mg/m^3) and in female rats at 161 or 496 ppm (968 or 2981 mg/m^3). Male and female B6C3F$_1$ mice were exposed to single oral doses of 310 or 638 mg/kg/day and inhalation concentrations of 158 or 501 ppm (950 and 3011 mg/m^3). Inhalation exposures were nose only and lasted 6 hours for both single and repeated exposures. Absorption was rapid via the digestive and respiratory tracts, with better absorption by the oral route than by inhalation exposure. B6C3F$_1$ mice demonstrated increased 1,4-DCB absorption relative to F344 rats after inhalation (59% in mice versus 25–33% in rats). However, absorption was similar via the oral route in F344 rats and B6C3F$_1$ mice (after single dose, 72% in rats and 71% in mice; after repeated exposure, 62% in rats). In this study, the dose levels, dose frequency, and sex did not have a large influence on the extent of absorption.

In another study, male and female F344 rats were exposed by inhalation to 500 ppm (3000 mg/m^3) for 24 hours to determine the organ distribution of 1,4-DCB (Umemura et al., 1990). Concentrations of 1,4-DCB in the serum, liver, kidney, and fatty tissues were measured by gas chromatography in groups of animals sacrificed at 6, 12, and 24 hours during exposure, and 3, 6, 12, and 24 hours after exposure. The peak concentration of 1,4-DCB in fatty tissues was about 100 times that in serum after the inhalation exposure. Following the 24-hour exposure to 500 ppm 1,4-DCB, the peak concentration reached almost 3 milligrams per gram (mg/g) of fatty tissue. However, the concentration declined to below 0.5 mg/g fat by 24 hours post exposure. There were no significant differences in the 1,4-DCB serum levels between male and female rats, although the concentrations in the livers of female rats were significantly higher than those of male rats. Conversely, significantly higher levels were found in the kidneys of male rats compared to female rats.

In a companion study, Umemura et al. (1989) observed higher organ-to-serum distribution ratios in liver and kidney in F344 rats exposed by inhalation to 1,4-DCB for 24 hours compared to animals receiving 1,4-DCB by oral gavage. The authors attributed this difference, particularly regarding the kidney-to-serum ratio, to the “first-pass” effect of orally absorbed 1,4-DCB passing through the liver and being metabolized prior to reaching other organs. With chronic exposure to 75 or 500 ppm (450 or 3000 mg/m^3), 5 hours/day, 5 days/week, adipose tissue levels of 1,4-DCB were considerably lower at 18 months compared to peak levels measured at 6 months (Bomhard et al., 1998). However, the results reported in these studies were inadequate for OEHHA to determine the amount of 1,4-DCB absorbed.

Elimination of radiolabeled 1,4-DCB following oral or inhalation exposure was mostly via the urine (>80%) and, to a lesser extent, the fecal and biliary pathways (Hawkins et al., 1980; Wilson et al., 1990; Hissink et al., 1996). Little of the radiolabel was excreted in expired air. When checked in bile-cannulated animals after a single dose, up to 63% of the excreted ^{14}C was in the bile. However, since less than 10% of the dose was eliminated in the feces, most of the radiolabeled product was likely reabsorbed and excreted via the urine (Hawkins et al., 1980). Repeated daily inhalation of ^{14}C-1,4-DCB showed that most of the ^{14}C was eliminated in the first 24 hours after cessation of exposure, but a small proportion could still be detected in urine on the fifth day after cessation of exposure (Hawkins et al., 1980).

Elimination of the ^{14}C-1,4-DCB absorbed dose was more complete after oral exposure than after inhalation exposure. Seven days following oral exposure, the mean cumulative total excretion was 80%–99% of the dose in F344 rats and male B6C3F$_1$ mice, as reported by Wilson et al. (1990). Klos and Dekant (1994) observed that within 72 hours of administration of 900 mg/kg ^{14}C-1,4-DCB, approximately 41% of the radioactivity was recovered from urine, and 6–8% was collected from feces. In

contrast, seven days after inhalation exposure, the mean cumulative total excretion was 35% in F344 rats and 55% in male B6C3F$_1$ mice. Of the total excreted, radioactivity in urine was 18%–32% in rats and 32%–47% in mice, while that in feces was and 2% in rats and 6%–19% in mice. The fraction of eliminated radiolabel in expired air was not determined. The percentage of ^{14}C-1,4-DCB excreted in the urine was not affected significantly by the dose (Wilson et al., 1990).

Pulmonary elimination after gavage administration accounted for less than 1% of the administered doses in two studies (Hawkins et al., 1980; Hissink et al., 1997a). However, up to 12% of the orally administered dose was eliminated via the lungs in the study by Wilson et al. (1990).

Metabolic pathways of 1,4-DCB

1,4-DCB is extensively metabolized, as shown by low or non-detectable levels of parent compound in the urine and feces in available studies. The metabolism of 1,4-DCB is depicted in Figure 1. Metabolism is believed to occur primarily in the liver and does not appear to depend on the route of administration (Hissink et al., 1997a).

Regardless of the route of absorption, the initial step in 1,4-DCB metabolism is mainly generation of an epoxide by CYP450 enzymes. In rats and mice, oxidation leads to the 1,2-epoxide and 2,3-epoxide (den Besten et al., 1992), whereas in humans, the 2,3-epoxide is the main product of metabolism (Bogaards et al., 1995). Hydrolysis of the epoxide was not a route of biotransformation in any species since no dihydrodiols were identified and no effect of cyclohexene oxide, an inhibitor of epoxide hydrolase, was observed (Hissink et al., 1997b). The epoxides can be further oxidized to mainly form 2,5-DCP and minor amounts of 2,4-DCP (Hawkins et al., 1980; den Besten et al., 1992). 2,5-DCP is considered to be the main metabolite of 1,4-DCB in both humans and rats (Pagnotto and Walkley, 1965; Angerer et al., 1992; Hill et al., 1995b; Yoshida et al., 2002b).

In rodent studies, dichlorophenols are primarily excreted in urine as sulfate and glucuronide conjugates, with lesser amounts (about 10%) excreted as GSH conjugates. Only small amounts of unconjugated dichlorophenols have been detected in urine of exposed animals (Hawkins et al., 1980; Hissink et al., 1996).

The dichlorophenols can be further oxidized to quinones and catechols (den Besten et al., 1992; Klos and Dekant, 1994). Both male and female F344 rats showed sulfate and glucuronide conjugates of 2,5-DCP and 2,5-dichlorohydroquinone (Klos and Dekant, 1994). Mercapturic acids were also excreted in the urine of rats (Klos and Dekant, 1994; Hissink et al., 1997a). Hissink et al. (1997a) reported that in male Wistar rats, 57%–63% of 1,4-DCB urinary metabolites was excreted as the 2,5-DCP sulfate and 19%–25% as the 2,5-DCP glucuronide. Another 10% of total urinary

metabolites were excreted as the GSH conjugates of the epoxide of 1,4-DCB, the mercapturic acid N-acetyl-cysteine-S-1,4-DCB, and its precursor, N-acetyl-cysteine-S-dihydro-hydroxy-1,4- DCB. In addition, after a single oral exposure for a week of 800 mg/kg 1,4-DCB to male Wistar rats, two sulfur containing metabolites, 2,5-dichlorophenyl methyl sulfoxide and 2,5-dichlorophenyl methyl sulfone, were found in the blood and urine (Kimura et al., 1979). However, their excretion in the urine was much less than that of the primary metabolite, 2,5-DCP.

In oral exposure studies, 1,4-DCB induced liver CYP dependent monooxygenases in a dose-dependent manner in both sexes of F344 rats at doses >380 mg/kg (Allis et al., 1992). In F344 male rats, oral doses of 75 to 300 mg/kg/day induced liver microsomal CYP at 1, 4, and 13 weeks of exposure (Lake et al., 1997). Induction of liver microsomal CYP also occurred in $B6C3F_1$ male mice at 600 mg/kg/day, but not at 300 mg/kg/day, during 1, 4, and 13 weeks of exposure. CYP was not increased in albino rats given 1,4-DCB via gavage at lower doses of 10, 20, and 40 mg/kg/day for 90 days (Carlson and Tardiff, 1976).

Several CYP enzymes are involved in the metabolism of 1,4-DCB including 2B1, 3A1 and 3A4, but the primary isoenzyme responsible for metabolism is CYP2E1 (Hawkins et al., 1980). In male Wistar rats, CYP2E1 induction via isoniazid increased the clearance rate of urinary 2,5-DCP and reduced the serum half-life of 1,4-DCB (Hissink et al., 1997a). Studies in microsomes from Wistar rats treated with CYP inhibitors show that both CYP2B1/2 and CYP2E1 are involved in the biotransformation of 1,4-DCB in rats (Hissink et al., 1997b).

CYP2E1 is the main P450 isozyme involved in the metabolism of 1,4-DCB by human liver microsomes (Bogaards et al., 1995; Hissink et al., 1997b; Nedelcheva et al., 1998). Microsomes from cell lines expressing human CYP1A1, 3A4, 2E1 and 2D6 incubated with 1,4-DCB were studied (Bogaards et al., 1995). CYP2E1 showed the highest rate of oxidation to produce 2,5-DCP. CYP2D6 showed low or non-detectable activity towards 1,4-DCB. Nedelcheva et al. (1998) observed that 1,4-DCB oxidation was inhibited by triacetyloleandomycine, a CYP3A1 inhibitor, in microsomes from human livers; this inhibition occurred to varying degrees, suggesting individual differences in 1,4-DCB catalysis. Nedelcheva et al. also showed that in human microsomes, CYP1A2, 2A6, 2B6, and 2C9 do not catalyze 1,4-DCB.

Fisher et al. (1990) reported that in liver slices from male Sprague-Dawley (SD) and F344 rats, the majority (>60%) of 1,4-DCB was found conjugated to GSH or as a cysteine conjugate, with small amounts of the sulfate detected (~10% of total metabolites). In human liver slices, the pattern was different, with GSH still being the predominant metabolite (~55%) but with an approximately equal distribution of glucuronide and sulfate conjugates (22%–24%).

Several species differences exist in the metabolism of 1,4-DCB. Hissink et al. (1997b) demonstrated the differences seen in biotransformation of radiolabeled 1,4-DCB *in vitro* in the hepatic microsomes of 3 strains of rats (F344, SD and Wistar), mice, and humans. Within the 3 strains of rats, the conversion of 1,4-DCB (% of total radioactivity) was similar in the microsomes from F344 and Wistar strains, whereas SD rats showed less biotransformation than the other two strains. Mice microsomes produced the most reactive metabolites as shown by covalent binding to macromolecules. This species difference is believed to be a factor in 1,4-DCB toxicity in mice, but not rats. The species rank order for total *in vitro* hepatic microsomal conversion of 1,4-DCB was mouse > rat >> human, with the human hepatic microsomes produced the least reactive metabolites.

Differences in metabolism between rats and humans were not observed in precision cut liver slices incubated with 1 mM 1,4-DCB (Fisher et al., 1995). 1,4-DCB was metabolized equally in liver slices of both rat strains (F344 and SD) and donated human liver slices. 1,4-DCB isomer produced an equal amount of glucuronide and sulfate conjugate in both rat strains and human liver slices. In addition, GSH and cysteine derivative conjugates were also formed in the rat and human liver slices. These GSH/cysteine metabolites were similar in the rat and human samples at the studied time points.

Nedelcheva et al. (1998) demonstrated that while microsomal oxidation was relatively less influenced by sex and species, significant differences in the formation of covalently-bound products were seen. Microsomes from female rats formed less covalently-bound products of 1,4-DCB than that of male rats and male and female mice. The studies in human liver microsomes also showed that the metabolic rates to soluble and covalently bound metabolites were lower than in rats and mice.

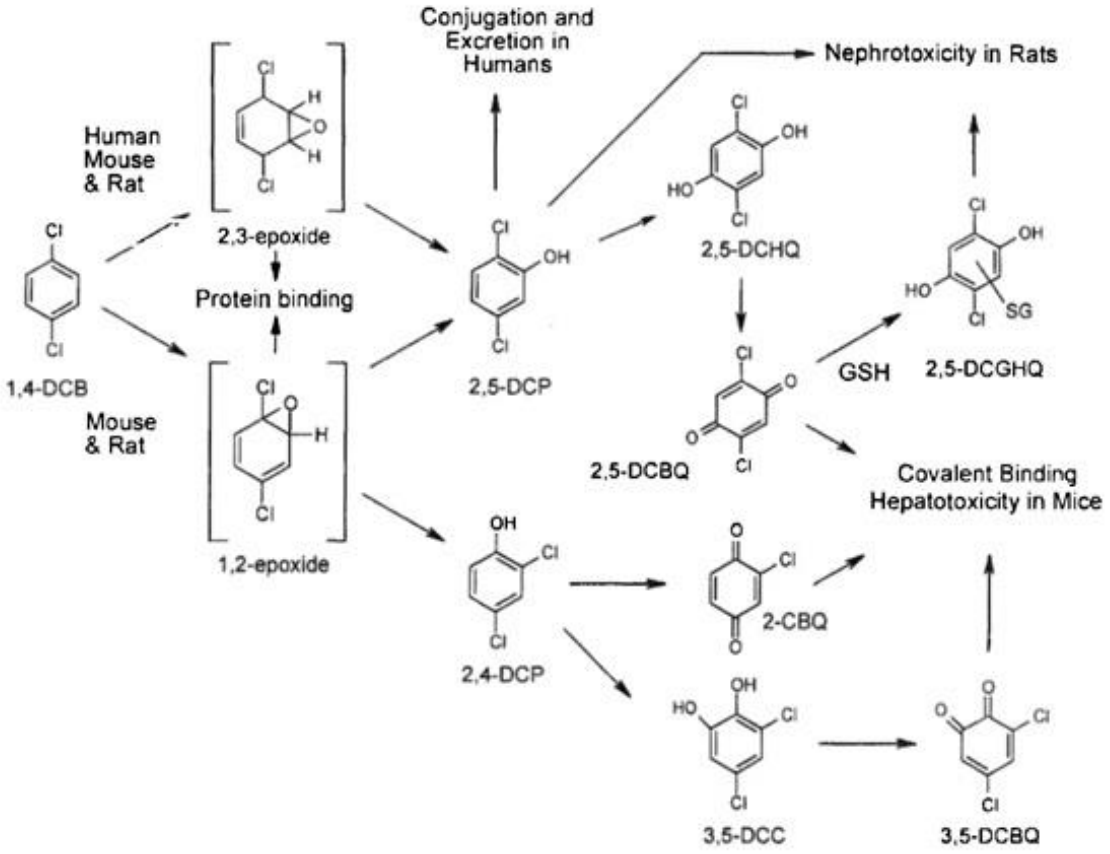

Figure 1. Proposed pathways for 1,4-DCB metabolism.

Source: Figure taken from Muller (2002) and modified by OEHHA. Pathways for the formation of reactive metabolites by mouse, rat and human microsomes and their proposed effects are shown. Abbreviations: CBQ – chlorobenzoquinone; DCB – dichlorobenzene; DCBQ – dichlorobenzoquinone; DCC – dichlorocatechol; DCGHQ – dichlorogluthionylhydroquinone; DCHQ – dichlorohydroquinone; DCP – dichlorophenol; GSH – glutathione; SG – glutathione-S-yl-metabolite

Physiologically based pharmacokinetic (PBPK) modeling of 1,4-DCB

Yoshida et al. (1998) studied the inhalation pharmacokinetics of 1,4-DCB in male SD rats using a compartmental model and a closed chamber system. Absorption of inhaled 1,4-DCB was measured using a linear four-compartment model including a chamber air compartment, a rat central compartment, a rat peripheral compartment, and an adsorption space compartment. Following injection of a specified amount of 1,4-DCB into the chamber air, the disappearance of 1,4-DCB from the chamber air followed linear kinetics, suggesting saturation kinetics was not attained at the concentration range studied. The rate constants derived from the experiment showed mainly partitioning of inhaled 1,4-DCB into the blood, and that once absorbed there is extensive distribution into the peripheral compartment (i.e., primarily fat). The calculated metabolic rate constant confirmed that metabolism is the predominant route of elimination for 1,4-DCB.

The toxicokinetics of 1,4-DCB in humans was also studied by Yoshida et al. (2002a). Continuous inhalation exposure by mouthpiece to 1,4-DCB at 2.5 ppm (15 mg/m^3) was carried out in 7 male subjects for 1 hour, following which 1,4-DCB concentrations were monitored in expired air and serum. 2,5-DCP, the urinary metabolite of 1,4-DCB, was monitored in the urine of the subjects. The toxicokinetics of 1,4-DCB was evaluated using a linear two-compartment model - a central (serum) compartment and a peripheral (fat, tissue, etc.) compartment. For each subject, the toxicokinetic parameters for biotransformation of 1,4-DCB were estimated by simultaneously fitting the concentration-time course data, obtained by analyzing urine and serum samples, to the linear two-compartment model. The mean calculated rate constant for distribution from the central to the peripheral compartment (k_1: 0.30 ± 0.08 h^{-1}) was higher than the rate constant for distribution from the peripheral to the central compartment (k_2; 0.060 ± 0.018 h^{-1}) and for metabolic elimination of 1,4-DCB (k_e; 0.022 ± 0.008 h^{-1}). This finding indicates that once absorbed, 1,4-DCB distributes rapidly to the peripheral compartments, demonstrating a high affinity for fat tissue. The calculated means of the apparent volumes of distribution for the central and peripheral compartments were 145 liters and 688 liters, respectively, again indicating 1,4-DCB is highly distributed to the peripheral compartment in humans.

For the individual time courses of urinary excretion, accurate fits were achieved for the simulation curves to the experimental data for each subject (Yoshida et al., 2002a). Based on the toxicokinetic analysis in the subjects, the serum steady state concentration of 1,4-DCB due to inhalation was calculated to be 3.5 ng/ml in humans chronically exposed to 1 ppb (6.01 µg/m^3) 1,4-DCB. Daily absorption due to chronic inhalation exposure to 1 ppb was estimated at 0.13 to 0.59 mg/day in the subjects, with a mean of 0.27 mg/day. In the previous inhalation toxicokinetic analysis in rats, Yoshida et al. (1998) calculated an absorption amount of 1.83 µg/day per kg in rats

chronically exposed to 1 ppb 1,4-DCB. When the authors extrapolated to 67 kg, the mean body weight of the human subjects in the Yoshida et al. (2002a) human study, the absorption amount (extrapolated to humans from the earlier rat study) was 0.12 mg/day, which agrees approximately with the mean absorption intake in the human study of 0.27 mg/day.

However, experimental data are lacking to parameterize a PBPK model for simulating organ dosimetry and reactive metabolites of 1,4-DCB in rats and humans.

4.2 Toxicokinetic and Biomonitoring Studies in Children and Adults

There is only one controlled inhalation exposure study by Yoshida et al. (2002a) available examining the toxicokinetics of 1,4-DCB in humans. However, an extensive number of general population and occupational biomonitoring studies have been carried out to determine the concentration of 1,4-DCB in human tissues and its metabolites in urine. 1,4-DCB has been found in the blood (Bristol et al., 1982; Hill et al., 1995a), urine (Pagnotto and Walkley, 1965; Ghittori et al., 1985; Hill et al., 1995a), adipose tissue, and breast milk (Jan, 1983) of participants in biomonitoring surveys and studies.

As noted in the PBPK modeling Section, Yoshida et al. (2002a) investigated the toxicokinetics of 1,4-DCB in seven adult human male volunteers exposed to a target concentration of 15 mg/m^3 (2.5 ppm) 1,4-DCB via mouthpiece for one hour. The pulmonary retention of 1,4-DCB in the subjects ranged from 46% to 67%, and the average was 56%. However, the 1,4-DCB concentration in exhaled air hardly varied among the subjects during exposure and decreased rapidly after exposure, falling below the detection limit within 10 minutes after the end of exposure. Therefore, the absorption rate of 1,4-DCB into the body through the pulmonary route was considered to be constant during exposure, and once absorbed into the blood, very little (percent not given) 1,4-DCB was excreted in the expired air of the tested subjects. Yoshida et al. (2002a) determined the amount of 2,5-DCP eliminated via urine for 9 to 11 hours after the beginning of the exposure period. During this time, only 5%–16% of the absorbed 1,4-DCB was eliminated indicating a significant time period (half-life not determined) is necessary for 1,4-DCB to be removed from the body.

Since the 1980s, periodic biomonitoring for chemicals in blood and urine of the US population has been conducted by CDC as part of the National Health and Nutrition Examination Survey (NHANES). Included in the survey is the biomonitoring for 1,4-DCB in blood and its metabolite, 2,5-DCP, in urine. Urinary concentration of 2,5-DCP is considered a reliable biomarker of 1,4-DCB exposure (Yoshida et al., 2002b). 2,5-

DCP was detected in 98.5% of the urine samples from the study participants in the 2007–2008 and 2009–2010 NHANES biomonitoring survey cycles.

In Table 1, the NHANES data show generally higher levels of the metabolite in the urine of children than in the population as a whole (CDC, 2022). However, urinary 2,5-DCP levels in adults have dropped greater than ten-fold between the 1988–1994 survey and the 2015–2016 survey. In children, 2,5-DCP urinary levels have dropped roughly 2-fold between the 2003–2004 survey and the 2015–2016 survey.

Table 1. Selected NHANES biomonitoring survey results for creatinine-corrected urinary 2,5-DCP.

Year	Age (years)[a]	Sample number	Geometric mean (µg/g Cr)	50th percentile (µg/g Cr)	95th percentile (µg/g Cr)	Source
<1987	2–6	197	ND	11	200	Hill, 1989
1988–1994	20–59	892	ND	24	670	Hill, 1995
2003–2004	All	2522	12.5	9.29	578	CDC, 2022
2011–2012	All	2487	4.8	3.19	215	
2013–2014	All	2684	2.77	1.82	108	
2015–2016	All	2650	3.02	2.03	133	
2003–2004	6–11	314	15.2	10.6	830	CDC, 2022
	12–19	720	12.7	9.05	549	
2005–2006	6–11	356	11.6	8.00	419	
	12–19	702	8.88	6.91	279	
2007–2008	6–11	389	11.5	7.70	420	
	12–19	401	8.79	5.56	353	
2009–2010	6–11	415	9.36	6.25	536	
	12–19	420	6.44	4.05	257	
2011–2012	6–11	395	5.01	3.02	377	
	12–19	388	4.04	2.41	157	
2013–2014	6–11	409	3.66	2.41	172	
	12–19	462	2.21	1.53	54.2	
2015–2016	3–5	140	5.95	3.51	440	
	6–11	415	4.92	3.07	224	
	12–19	405	3.98	2.61	235	

[a] The notation "All" refers to the total study population.

Abbreviations: CDC – United States Centers for Disease Control and Prevention, The; 2,5-DCP – 2,5-dichlorophenol; µg/g Cr – micrograms per gram of creatinine; ND – no data; NHANES – National Health and Nutrition Examination Survey.

Biomonitoring of 1,4-DCB in blood of the general population was also conducted by NHANES (CDC, 2022) and the results are shown in Table 2. 1,4-DCB in blood was below the limit of detection (LOD) of 0.04 µg/L (i.e., 0.040 ng/mL) in most blood samples. However, from 2011–2012 to 2017–2018, a greater than two-fold drop in 1,4-DCB blood levels occurred in the 75th and 90th percentiles for all participants, and for the subset of adolescents/young adults aged 12–19. The survey in adults only from 1988–1994 suggest that 1,4-DCB blood levels in the 90th percentile may have decreased in adults more than 10-fold following the 2017–2018 survey.

Table 2. Selected NHANES biomonitoring survey results for 1,4-DCB in blood.

Year	Age (years)	Sample number	Geo–metric Mean (ng/mL)	Median (ng/mL)	75th percentile (ng/mL)	90th percentile (ng/mL)	Source
1988–1994	20–59	954	ND	0.33 (m) 0.30 (f)	ND	3.89 (m) 4.83 (f)	Hill et al., 1995
2011–2012	All[a]	2709	*	<LOD[b]	0.143	0.670	CDC, 2022
2017–2018	All[a]	2855	*	<LOD[b]	0.064	0.242	CDC, 2022
2011–2012	12–19	501	*	<LOD[b]	0.144	0.543	CDC, 2022
2017–2018	12–19	474	*	<LOD[b]	0.061	0.218	CDC, 2022

[(a)] The notation “All” refers to the total study population.
[(b)] LOD (Limit of Detection) = 0.040 ng/mL for 2011 to 2018 NHANES surveys; 0.07 ng/mL for 1988–1994 NHANES III reported in Hill et al. (1995).
* Not calculated since the proportion of results below LOD was too high to provide a valid result.
Abbreviations: CDC – United States Centers for Disease Control and Prevention; ND – data not determined or not presented; m – males; f – females; ng/mL – nanograms per milliliter.

In addition to the ongoing NHANES biomonitoring analyses, other studies looked for correlations between 1,4-DCB in blood and 2,5-DCP in urine (Hill et al., 1995a,b), and correlations between airborne exposure to 1,4-DCB and 1,4 DCB in blood (Lin et al., 2008; Sexton et al., 2005) or 2,5-DCP in urine (Yoshida et al., 2002b; Yoshida et

al., 2021; Pagnotto and Walkley, 1965; Ghittori et al., 1985), and are summarized below.

Blood and urine samples were collected from a subset of adults (age 20–59 years old) participating in the 1988–1994 NHANES III to look for correlations between 1,4-DCB in blood and its metabolite, 2,5-DCP, in urine (Hill et al., 1995a; Hill et al., 1995b). Ninety-eight percent of participants had detectable levels of 2,5-DCP in their urine, and 96% had detectable levels of 1,4-DCB in their blood. Among 694 participants, a strong correlation was found between urinary 2,5-DCP and the blood concentration of 1,4-DCB (Pearson correlation coefficient = 0.82, $p < 0.0001$). Neither age nor gender was related to creatinine-corrected urinary 2,5-DCP or blood 1,4-DCB.

The blood concentration of 1,4-DCB was also found to be correlated with 2–3 day personal airborne exposure to 1,4-DCB. Samples of blood taken from 354 persons 20–59 years of age in the 1999–2000 NHANES survey were analyzed for 1,4-DCB and other VOCs (Lin et al., 2008). The concentration of VOCs in ambient air was measured using badge-type organic vapor monitors worn by the participants for 48–72 hours. At the return of the monitors, whole blood samples were drawn. Air samples and blood samples were analyzed using gas chromatography/mass spectrometry (GC/MS). In non-smokers, the geometric mean (GM) concentration of 1,4-DCB in blood was 0.235 ng/ml and the GM concentration in ambient air was 3.57 $\mu g/m^3$ (0.59 ppb). In smokers, The GM concentration of 1,4-DCB in blood (0.270 ng/ml) and airborne 1,4-DCB (2.24 $\mu g/m^3$ (0.37 ppb)) were only marginally different from that of non-smokers. Significant associations between blood and airborne 1,4-DCB was found for the unadjusted regression models for smokers (R^2 = 0.37) and non-smokers (R^2 = 0.68). Adjusting the models for covariates such as age, gender, body mass index, race/ethnicity and alcohol consumption did not affect the relationship between levels of 1,4-DCB in air and blood (adjusted regression model R^2 = 0.46 for smokers and 0.72 for non-smokers).

Sexton et al. (2005) showed in a smaller survey of children (n = 150, age 6–10 years old) that personal exposure to airborne 1,4-DCB levels did not vary greatly between sampling days. This could explain the strong association between blood and air levels of 1,4-DCB (relative to other VOCs examined) observed by Lin et al. (2008), since air concentrations of 1,4-DCB are collected over 2–3 days, and blood levels tend to reflect more recent exposure immediately before blood collection. In this study conducted in two minority neighborhoods in Minneapolis, MN, a strong statistical association between two-day personal exposure and blood concentration was found for 1,4-DCB (R^2 = 0.79). The overall GM blood concentration of 1,4-DCB was 0.242 ng/ml, similar to the concentration found in adults in the study by Lin et al. (2008).

Yoshida et al. (2002b) examined the association between airborne 1,4-DCB exposure and urinary 2,5-DCP in 119 adult individuals selected from the general population in Osaka, Japan. Personal exposure concentrations of 1,4-DCB were determined for a 24-hour period (7 am to 7 am the next morning) and urine was collected at the end of the exposure period. The GM air concentration of 1,4-DCB was 3.5 ppb (21.0 µg/m^3) and the creatinine-corrected GM 2,5-DCP level was 0.46 mg/g creatinine. The Pearson correlation coefficient between 1,4-DCB exposure and urinary 2,5-DCP was 0.81 ($p < 0.001$), indicating a strong association between these values.

Yoshida et al. (2021) also conducted a biomonitoring study in Japanese children (age 6–15 years old) to examine the relationship of indoor exposure to 1,4-DCB and urinary 2,5-DCP. Fixed air monitors were placed in 68 bedrooms of 112 children (some siblings shared a bedroom) and collected 24 hour air samples. The geometric mean airborne concentration of 1,4-DCB was 5.2 µg/m^3 (0.87 ppb) and the range was 0.57 to 462 µg/m^3 (0.09 and 77 ppb). The detection frequency in the bedrooms was 100%. The main source was suggested to be moth repellents containing 1,4-DCB. The first morning urine void was collected from the children on the day that the bedroom air was monitored. The geometric mean concentration of urinary 2,5-DCP was 12 µg/g creatinine, with a range of 1.8 to 615 µg/g creatinine (detection frequency 100%). A significant correlation was found between the airborne concentration of 1,4-DCB in their bedroom and the urinary excretion of creatinine-corrected 2,5-DCP ($p < 0.05$, $r = 0.757$). The geometric mean daily intake was calculated to be 3.6 mg/kg BW/day. The overall median inhalation absorption amount as compared to the overall absorption amount of 1,4-DCB was estimated to be 30%, with inhalation as the main route of exposure in children exposed to high levels of 1,4-DCB (>240 µg/m^3 or 40 ppb). Ingestion of house dust contaminated with 1,4-DCB was also considered to be an important exposure pathway in children.

Urinary levels of 2,5-DCP in workers have also correlated with airborne exposure to 1,4-DCB in the workplace. Higher 1,4-DCB exposure, and subsequent urinary 2,5-DCP levels, were much higher than levels found in the general population. Occupational exposure of 9 to 34 ppm (54 to 204 mg/m^3) 1,4-DCP resulted in urinary level of 20 to 91 mg/L 2,5-DCP (Pagnotto and Walkley, 1965). On the other hand, lower exposures of 3.5 ppb (0.0035 ppm) 1,4-DCB in the general population have resulted in lower urine concentrations of 0.52 mg/L 2,5-DCP (0.46 mg/g creatinine-corrected) (Yoshida et al., 2002b).

Ghittori et al. (1985) used personal samplers to determine the daily 8-hour time-weighted average (TWA) 1,4-DCB exposures in four chemical factory workers over a 5-day workweek. Urine samples were collected before and after work each day and the concentration of 2.5-DCP were determined in each sample. A significant

correlation (r = 0.64) was found between the difference in 1,4-DCB concentration at the beginning and the end of the workday and the air concentration of 1,4-DCB. The 8-hour TWA concentration ranged from 24.93 mg/m^3 to 77.79 mg/m^3 (4.15 to 12.94 ppm). The difference between morning and afternoon urinary 2,5-DCP levels ranged from 17.50 µg/L to 55,90 µg/L. There was a tendency for the morning 2,5-DCP concentration in urine to increase during the workweek, suggesting accumulation of 1,4-DCB in the body during the week.

5. Acute Toxicity of 1,4-Dichlorobenzene

5.1 Acute Toxicity to Adult Humans

In this section, exposure durations are limited to approximately two weeks or less, which is the duration that has been used to define acute/subacute exposures in toxicology study protocols. Currently, there is very limited information on acute 1,4-DCB exposures of ≤24 hours in humans.

5.1.1 Case reports

A few case reports of toxic effects resulting from acute exposure to 1,4-DCB in adults and children were found in the literature. These reports lack dose-response information and verification that exposure to other toxic or infectious agents had not occurred. Case reports of acute toxicity in children are reported below in Section 5.2.

In a case report, acute allergic purpura, dyspnea, and kidney damage secondary to acute allergic purpura was reported in an elderly man following acute exposure to 1,4-DCB (Nalbandian and Pearce, 1965). Symptoms began while sitting in a chair that was treated with 1,4-DCB crystals earlier in the day, and he was admitted to the hospital 24-48 hours after the exposure. The patients' blood urea nitrogen (BUN) level rose to 57 mg/100 cc (57 mg/dL) on the fourth day of hospitalization but fell below 15 mg/100 cc (15 mg/dL) on the 18th day of hospitalization. BUN levels above the normal range of 8–20 mg/dL for adult men is suggestive of kidney damage. The patient's condition improved, and he was discharged on the 31st hospital day. Indirect basophil degranulation testing with the patient's serum still indicated sensitivity to 1,4-DCB five months after initial exposure. No estimation of the airborne concentration is mentioned in the publication, but the description of the exposure indicates dermal exposure also occurred.

5.1.2 Occupational Studies

Health surveys and examinations were conducted on 58 men who were intermittently exposed occupationally to 1,4-DCB for an average of 4.75 years (range of 8 months to 25 years) (Hollingsworth et al., 1956). The facility where the surveys took place

was not explicitly described but involved the manufacture and handling of 1,4-DCB. Potential exposure to other VOCs was not described, although the authors indicated co-exposure to naphthalene did not occur.

In the first survey, analysis of 62 spot samples of workroom atmospheres showed that there was faint odor at 15–30 ppm (90–180 mg/m^3), strong odor at 30–60 ppm (180–360 mg/m^3), and painful irritation of eyes and nose at 80–160 ppm (481–962 mg/m^3). These observations suggest recurrent acute exposures to airborne 1,4-DCB result in sensory irritation (Table 3). In a second survey, workers described exposure to concentrations of 100–725 ppm (601–4357 mg/m^3) with an average of 380 ppm (2284 mg/m^3) as uncomfortable, with some wearing respirators. The particular job that resulted in this concentration range was not described. Unacclimated individuals could not tolerate this concentration without wearing a respirator. At exposure to 5–275 ppm (30–1653 mg/m^3) with an average of 90 ppm (541 mg/m^3) workers did not complain of discomfort. The authors noted that workers can become acclimated to the sensory irritant effects of 1,4-DCB following repeated occupational exposure and can tolerate concentrations that unacclimated persons will not tolerate.

A third survey was conducted after revision of operating procedures that resulted in lower concentrations of 1,4-DCB in the air. However, there was an increase in complaints of eye and nasal irritation by the workmen after the changes were made. Under conditions which arose during such complaints, 21 air samples showed 1,4-DCB levels from 50–170 ppm (301–1022 mg/m^3), with an average of 105 ppm (631 mg/m^3). Twenty-five air samples collected under conditions in which there were no complaints were in the range of 15–85 ppm (90–511 mg/m^3), with an average of 45 ppm (270 mg/m^3). The authors concluded that painful irritation of the eyes and nose was usually experienced at 50–80 ppm, although the irritation threshold was higher (80–160 ppm) in workers acclimated to exposure. No description of unacclimated persons exposed to 1,4-DCB was included in the report. Additional data on blood counts and eye examinations from these surveys are noted in Section 6.1 (Chronic Toxicity to Adult Humans).

Table 3. Occupational 1,4-DCB exposure levels resulting in sensory irritation conducted by Hollingsworth et al. (1956).

Survey	Notes	Exposure Concentration	Results
1st survey	62 air samples collected, average concentration 85 ppm with a range of 10–550 ppm	15–30 ppm	Faint odor
		30–60 ppm	Strong odor
		80–160 ppm	Painful irritation of the eyes and nose
		>160 ppm	Irrespirable for unacclimated persons
2nd survey	Unspecified time after the 1st survey using the same equipment and operating procedures	Average concentration: 380 ppm Range: 100–725 ppm	15 samples collected, uncomfortable for acclimated persons, some workers used respirators. Not tolerable by unacclimated persons, needed gas mask
		Average concentration: 90 ppm Range: 5–275 ppm,	32 samples collected, considered acceptable to acclimated workmen
3rd survey	After revision of operating procedures and equipment	Average concentration: 105 ppm Range: 50–170 ppm	21 air samples collected due to complaints of eye and nasal irritation by workmen
		Average concentration: 45 ppm Range: 15–85 ppm	25 air samples collected, no complaints

Abbreviations: ppm – parts per million

There are several deficiencies in the Hollingsworth et al. study such as the limited experimental design, lack of individual exposure data, and the observations which only provide qualitative evidence of exposure-related sensory irritation. In addition, concentration data is listed as concentration ranges with median values, in which peak exposure concentrations cannot be determined (results of spot samples of atmosphere) and therefore a clear quantitative correlation between concentration and the sensory irritant effects cannot be corroborated.

Field studies to determine 1,4-DCB exposure and possible toxic effects in workers were carried out in three industrial plants manufacturing or handling 1,4-DCB (Pagnotto and Walkley, 1965). This study also examined the association with the urinary levels of 1,4-DCB and 2,5-DCP in the exposed workers (this information is presented in Section 4.2). 1,4-DCB air samples were collected on silica gel for approximately 10 minutes at a rate of 2.5 liters per minute. The number of air samples collected, and the number of workers at each plant were not clearly specified. The highest exposures were found in the chemical manufacturing plant with average concentrations of 24–34 ppm (144–204 mg/m^3), depending on the job [overall range: 7–49 ppm (42–294 mg/m^3)]. The distinct odor of 1,4-DCB was present at the manufacturing plant, but no painful irritation of the eyes or nose was reported by the workers except when there was direct contact with the crystals. In the other two plants, the odor was just detectable, and no discomfort was experienced by the workers. The average concentrations at these two facilities were between 7–25 ppm (42–150 mg/m^3), depending on the task. In the chemical manufacturing plant, 1,2-dichlorobenzene was also present at concentrations as high as 25% of the measured concentration for 1,4-DCB.

5.2 Acute Toxicity to Infants and Children

Acute hemolytic anemia, methemoglobinemia, and jaundice was reported in a 3 year-old boy after playing with “demothing” crystals containing 1,4-DCB for 4–5 days (Hallowell, 1959). Based on the case report, it is possible that ingestion, inhalation, and dermal exposure to 1,4-DCB occurred during the play. The boy showed severe hemolysis and required blood transfusion. According to the report, he recovered completely. Trace amounts of the 1,4-DCB metabolite 2,5-dichloroquinol (i.e., 2,5-dichlorohydroquinone) and two other unidentified phenols were found in the urine, but 2,5-DCP was not found. It was not explicitly stated in the report if the demothing product contained other chemicals, such as naphthalene.

Reichrtova et al. (1999) collected placenta samples from term deliveries in industrial and rural regions of Slovakia to analyze for selected organochlorine compounds. Specimens of cord blood from 2,050 neonates were simultaneously collected for determination of levels of total immunoglobulin E (IgE), a sensitive predictor of the risk for atopy, which is the tendency to produce an exaggerated IgE immune response to otherwise harmless environmental substances. Comparisons between regions revealed that both the placental contamination with 16 of 21 organochlorine compounds and the cord serum IgE levels were significantly higher in the industrial region. The combined concentration of 1,4- and 1,3-DCB in placental samples were higher than most of the organochlorine compounds investigated. Comparisons between regions revealed that both the placental contamination with 16 of 21 organochlorine compounds and the cord serum IgE levels were significantly higher in

the industrial region. Overall, Reichrtova et al. (1999) suggest an association between organochlorine compounds and the higher levels of total IgE in newborns, signaling a higher potential for allergic sensitization in industrial regions. No definitive conclusion regarding a relationship between 1,4-DCB exposure and cord blood IgE levels can be made from this study because there was exposure to many other organochlorine chemicals.

Delfino et al. (2003) analyzed VOCs in exhaled breath of 21 children with mild asthma that lived near major freeways in southern California. Eight VOCs, including 1,4-DCB, were measurable in >75% of breath samples obtained. Symptom diaries were filled out and peak expiratory flow maneuvers conducted daily over an approximate three-month period. Breath samples were collected on asthma-episode and symptom-free days. The observed mean exhaled breath concentration of 1,4-DCB was 36.29 µg/m^3 (6.04 ppb) with a range of 0.16–490.76 µg/m^3 (0.03–81.66 ppb). Twenty-four-hour outdoor air monitoring samples were also collected at a central site during the examination period. The mean ambient outdoor concentration of 1,4-DCB was 0.96 µg/m^3 (0.16 ppb), with 27% of samples below the limit of detection. However, neither exhaled breath nor ambient concentrations of 1,4-DCB were significantly associated with asthmatic symptoms.

5.3 Acute Toxicity to Experimental Animals

This section includes summaries of studies that used exposure durations of approximately 2 weeks or less. A summary table (Table 5) is included at the end of the section.

Tremors, weakness, eye irritation and unconsciousness were reported in rats, guinea pigs, and rabbits with daily 8-hour, 5 days/week exposures to an average concentration of 798 ppm (4796 mg/m^3) 1,4-DCB (Hollingsworth et al., 1956). The exposures ranged from 1 to 69 days in rats, 1 to 23 days in guinea pigs, and 1 to 62 days in rabbits. It was not explicitly stated when the signs of neurological and sensory irritant effects were first observed but may have begun in the first days or weeks of exposure. Daily observations of animals exposed to 341 ppm (2049 mg/m^3) 1,4-DCB for 7 hours/day, 5 days/week did not result in any apparent signs of toxicity.

In the two-generation study by Tyl and Neeper-Bradley (1989) tremor and perinasal/perioral encrustation were observed in most or all male and female rats ($p < 0.01$), often beginning on the first day of 6-hour 1,4-DCB exposures to animals in the high exposure group (Table 4). The average exposure concentration of the high exposure group over the duration of the study was 538 ppm (3233 mg/m^3). However, the initial analytical method was found to underestimate the vapor concentrations in the exposure chambers during the first 80 days of the study. The corrected mean

analytical concentration for the first day of exposure was 571 ppm, which is the more accurate exposure concentration producing acute effects starting on the first exposure day. Other signs of toxicity were observed in a significant number of the high exposure group animals ($p < 0.05$) with repeated exposures over days or weeks, including unkempt body appearance, salivation, hypoactivity, ataxia, and twitching.

Six-hour exposures of groups of rats to average concentrations of 66 or 211 ppm (397 or 1268 mg/m^3) 1,4-DCB during the two-generation study produced no significant clinical observations (Tyl and Neeper-Bradley, 1989). The corrected mean analytical concentrations for the 66 and 211 ppm groups on the first day of exposure was 67.8 and 207 ppm, respectively.

Table 4. Clinical observations of acute 1,4-DCB toxicity during the two-generation inhalation reproductive/developmental study.

Effect	Animals	0 ppm Number[a] (days)[b]	66 ppm Number (days)	211 ppm Number (days)	538 ppm Number (days)
Tremor	F_0 males	0	0	1 (10)	28** (1–83)
	F_0 females	0	0	0	28** (1–133)
	F_1 males	0	0	0	22** (0–85)
	F_1 females	0	0	0	20** (0–130)
Unkempt body	F_0 males	2 (82–85)	0	0	27** (73–106)
	F_0 females	0	0	0	27** (69–133)
	F_1 males	0	0	2(28)	28** (8–110)
	F_1 females	1(125)	0	1(121–122)	28** (2–143)
Periocular encrustation (in both eyes)	F_0 males	0	2 (4–10)	0	8** (3–106)
	F_0 females	2 (78–97)	0	1(89)	10* (1–79)
	F_1 males	2 (8–114)	3 (2–114)	1(8–114)	10 (0–112)
	F_1 females	0	1 (128)	0	10** (0–132)

[a] Number of animals exhibiting the findings at least once during the study. A total of 28 animals per sex were examined in each exposure group.

[b] Number of animals exhibiting the findings at least once during the specified range of days.

* and ** – Statistically significant from control group at $p < 0.05$ and $p < 0.01$, respectively, using Fishers exact test, as designated in the study report.

Abbreviations: F_0 – parental generation; F_1 – first generation; ppm – parts per million.

706 **Table 4. Clinical observations of acute 1,4-DCB toxicity during the two-**
707 **generation inhalation reproductive/developmental study (continued).**

Effect	Animals	0 ppm Number[a] (days)[b]	66 ppm Number (days)	211 ppm Number (days)	538 ppm Number (days)
Perinasal encrustation	F_0 males	6 (1–103)	10 (1–102)	6 (1–92)	19** (1–88)
	F_0 females	2 (78–95)	2 (64–66)	1 (102)	4 (1–82))
	F_1 males	3 (29–87)	5 (43–79)	3 (28–95)	4 (0–42)
	F_1 females	0	0	0	6* (0–121)
Salivation	F_0 males	0	0	0	8** (11–82)
	F_0 females	0	0	0	8** (8–121)
	F_1 males	no data	no data	no data	no data
	F_1 females	no data	no data	no data	no data
Perioral encrustation	F_0 males	0	1 (5)	0	25** (1–106)
	F_0 females	1 (68)	0	0	22** (1–112)
	F_1 males	1 (30–31)	0	2 (29–34)	24** (1–93)
	F_1 females	0	0	0	21** (1–44)
Hypoactive	F_0 males	1 (82)	0	0	7 (71–102)
	F_0 females	1 (50)	0	1 (102)	3 (50–97)
	F_1 males	0	0	0	8** (22–53)
	F_1 females	no data	no data	no data	no data
Ataxia	F_0 males	0	0	0	2(78–101)
	F_0 females	1 (50)	0	1 (102)	1 (118–121)
	F_1 males	0	0	0	9** (17–30)
	F_1 females	0	0	0	1 (13)

708 [(a)] Number of animals exhibiting the findings at least once during the study. A total of 28
709 animals per sex were examined in each exposure group.

710 [(b)] Number of animals exhibiting the findings at least once during the specified range of
711 days.

712 * and ** – Statistically significant from control group at $p < 0.05$ and $p < 0.01$,
713 respectively, using Fishers exact test, as designated in the study report.

714 Abbreviations: F_0 – parental generation; F_1 – first generation; ppm – parts per million.

Table 4. Clinical observations of acute 1,4-DCB toxicity during the two-generation inhalation reproductive/developmental study (continued).

Effect	Animals	0 ppm Number[a] (days)[b]	66 ppm Number (days)	211 ppm Number (days)	538 ppm Number (days)
Twitch	F_0 males	0	0	0	3 (78–81)
	F_0 females	no data	no data	no data	no data
	F_1 males	0	0	0	6* (8–34)
	F_1 females	0	0	0	1 (12)
Lacrimation (both eyes)	F_0 males	0	0	0	2 (1–79)
	F_0 females	no data	no data	no data	no data
	F_1 males	0	0	0	5 (12–31)
	F_1 females	0	0	0	8** (0–120)

[(a)] Number of animals exhibiting the findings at least once during the study. A total of 28 animals per sex were examined in each exposure group.

[(b)] Number of animals exhibiting the findings at least once during the specified range of days.

* and ** – Statistically significant from control group at $p < 0.05$ and $p < 0.01$, respectively, using Fishers exact test, as designated in the study report.

Abbreviations: F_0 – parental generation; F_1 – first generation; ppm – parts per million.

Groups of male F344 rats were exposed whole body to 1,4-DCB in air for 24 hours at concentrations of 0, 125, or 500 ppm (0, 700, or 3000 mg/m^3, respectively) to explore the relation of organ distribution of 1,4-DCB and liver and kidney toxicity (Umemura et al., 1989). Details specific to the toxicokinetics of 1,4-DCB from this study can be found in Section 4.1. Organ distribution and toxicity by the inhalation route was compared to other groups of male F344 rats given a single oral dose of 0 or 300 mg/kg 1,4-DCB in corn oil via gavage. Rats in the inhalation study were sacrificed at 6, 12, and 24 hours during exposure, and 3, 6, 12 and 24 hours after cessation of exposure. Rats in the oral study were sacrificed at 6, 12, 18, 24 and 48 hours after dosing.

Peak serum concentrations were highest in rats orally administered 1,4-DCB, but the Area Under the Curve (AUC) for serum, liver, kidney, and fat were greatest in rats exposed to 500 ppm 1,4-DCB by the inhalation route (Umemura et al., 1989). BUN was significantly increased ($p < 0.01$) in both 125 ppm and 500 ppm exposure groups but it was not increased after an oral dose of 300 mg/kg. In addition, hepatic but not

renal glutamate oxaloacetate transaminase (also known as aspartate transaminase; AST) and glutamate pyruvate transaminase (also known as alanine transaminase; ALT) were significantly increased ($p < 0.01$) after the inhalation exposure but not after the oral dose of 1,4-DCB.

In the kidney proximal tubules of rats in the inhalation study and in the gavage study, epithelial cell swelling, eosinophilic bodies and desquamation were seen and were greatest in the 500 ppm inhalation group. The kidney histopathological findings in rats exposed to 125 ppm by inhalation or 300 mg/kg by the oral route were similar in severity. The authors suggested that the severity of kidney damage was related to the kidney/serum dose ratios, which was greatest in the 500 ppm exposure group (ratio roughly averaging 7–8 from 0 to 24 hours after exposure) and similar between the 125 ppm exposure group and the oral dose group (ratios roughly averaging 4 over inhalation time scale 0 to 24 hours after exposure).

In a companion study by Umemura et al. (1990) that appeared to be run concurrently with the male rat study, female F344/DuCrj rats were exposed to 500 ppm (3005 mg/m^3) 1,4-DCB for 24 hours to compare organ distribution and kidney and liver effects with male rats also exposed to 500 ppm for 24 hours. Serum levels during exposure, and followed up to 24 hours post-exposure, were similar in both male and females. However, the peak concentration of 1,4-DCB in the liver was significantly higher in female rats, while the peak concentration of 1,4-DCB in the kidney was significantly higher in male rats. Eosinophilic bodies and desquamation of tubule epithelium were seen in male F344 rats sacrificed 24 hours after termination of exposure, but not in the females. Vacuolization in hepatocytes was seen in female F344 rats but not in male rats. The authors concluded that there are sex-related differences in the acute toxicity of 1,4-DCB in rats that are related, in part, to organ distribution of 1,4-DCB.

765 **Table 5. Summary of acute and subacute effects of 1,4-DCB inhalation**
766 **exposure in experimental animals.**

Reference	Animal model and exposure	Results	Point of Departure
Hollingsworth et al. (1956)	Rats (N=10) and guinea pigs (N=8) exposed to 0, 96, 158, 173, 341, 798 ppm for 8 hours/day, 5 days/week for 1 to 69 days (rats) or 1 to 23 days (guinea pigs) Rabbits (N=1) exposed to 0, 96, 158, 173, 798 ppm for 8 hours/day, 5 days/week for up to 62 days	Tremors, weakness, eye irritation and unconsciousness at 798 ppm beginning in first days or weeks of the study	NOAEL: 341 ppm (rats and guinea pigs) or 173 ppm (rabbits) LOAEL: 798 ppm based on signs of neurotoxicity and sensory irritation
Tyl and Neeper-Bradley (1989)	Rats (N=28 per sex) exposed to 0, 66, 211 and 538 ppm for 6 hours/day, 7 days/week for 15 (males) or 20 (females) weeks	Tremors, perinasal/perioral encrustation, and unkempt body appearance on first day of exposure at 538 ppm	NOAEL: 211 ppm LOAEL: 538 ppm based on signs of neurotoxicity and sensory irritation
Umemura et al. (1989)	Male rats (N=25 per group) exposed to 0, 125 or 500 ppm for 24 hours	↑ BUN and hepatic glutamate oxaloacetate transaminase and glutamate pyruvate transaminase at 125 and 500 ppm ↑ kidney proximal tubule damage that was dose-dependent	NOAEL: NA LOAEL: 125 ppm based on ↑ enzymes indicating liver and kidney damage, and microscopic evidence of kidney damage
Umemura et al. (1990)	Male and female rats (N=25 per sex) exposed to 0 or 500 ppm for 24 hours	Kidney tubule damage in male rats, and hepatocyte damage in female rats at 500 ppm	NOAEL: NA LOAEL: 500 ppm based on liver and kidney damage

767 Abbreviations: ↑ – increased significantly ($p < 0.05$) relative to control; BUN – blood urea
768 nitrogen; LOAEL – Lowest Observable Adverse Effect Level; N – number; NA – not applicable
769 NOAEL – No Observable Adverse Effect Level; ppm – parts per million.

6. Chronic Toxicity of 1,4-Dichlorobenzene

6.1 Adult Humans

6.1.1 Case Reports

Numerous case reports of subchronic/chronic human poisoning resulting from oral and/or inhalation exposure to 1,4-DCB are available in the literature. Most early reports noted severe liver damage as the most significant injury. However, later case studies found central nervous system (CNS) toxicity and dermatitis as the main effects, with little or no apparent liver injury. These reports lack information on the dose of 1,4-DCB resulting in subchronic or chronic injury and/or verification that exposure to other toxic agents had not occurred. Naphthalene is also used in mothball products and may have contributed to some of the effects (e.g., liver toxicity and anemia) observed in early reports of injury.

Cotter (1953) reported on four cases in which patients were exposed to high concentrations of 1,4-DCB for months to years. The airborne concentration was not determined in these cases, but the odor of 1,4,DCB in work spaces or homes was described as quite prominent in three cases and the room air was described as being saturated by 1,4-DCB vapor in the other. In one patient, an adult male, yellow atrophy, and cirrhosis of the liver was seen due to exposure to 1,4-DCB in his trade of caring for raw furs for two years; however, benzene poisoning was also suspected in this case. In another case, a female sales clerk working in a department store while exposed to open cans of 1,4-DCB for many months also exhibited yellow atrophy and cirrhosis of the liver. The sales clerk also exhibited dry skin, and jaundiced eyes and skin. Cotter et al. also reported the case of a man and his wife who were exposed to vapors from mothballs in their home for 3 to 4 months, and later died from acute yellow atrophy within one year of initial exposure. The man experienced numbness, clumsiness, and a burning sensation in the legs. Among the four patients described by Cotter (1953), anemia, or borderline anemia, was also present in two patients. Some other symptoms observed include jaundice with elevation of serum bilirubin in all cases, and elevated serum alkaline phosphatase present in three of the cases. Urinalysis showed “disturbances” of serum protein in all cases, and high non-protein nitrogen in two cases.

In more recent case reports and reviews, subchronic/chronic ingestion and/or inhalation of 1,4-DCB likely resulted in nonspecific tissue damage to the white matter of the brain leading to functional neurological decline (Dubey et al., 2014; Zhang and Moreno, 2014; Weidman et al., 2015; Pisano et al., 2019; Alaufi et al., 2020; Leong et al., 2020). This disorder, known as leukoencephalopathy, can be caused by a variety of different agents, including exposure to some environmental and industrial

chemicals such as 1,4-DCB. Symptoms include limb weakness, tremor, cog wheel rigidity, hypotonia (low muscle tone) and difficulty walking. Dysarthria (i.e., slow speech or mutism) was also found in some cases, as was bradyphrenia (slowed thinking and processing of information) and cognitive decline.

Exposure durations in these recent case reports (i.e., since the Cotter (1953) case reports), when known, were two months to as long as 21 years. Exposure was often due to habitual abuse of products containing 1,4-DCB. Withdrawal from exposure subsequent to hospitalization resulted in more severe symptoms in some cases. Another common disorder of subchronic/chronic 1,4-DCB exposure was dermatitis characterized as hyperkeratotic, hyperpigmented plaques (Dubey et al., 2014; Zhang and Moreno, 2014; Pisano et al., 2019; Alaufi et al., 2020). Anemia has also been found in a few reports, although it was unclear if this could have been a pre-existing condition unrelated to 1,4-DCB exposure. In many cases, exposure was confirmed by the finding of 1,4-DCB in blood or 2,5-DCP in urine. In reports that included follow-up visits after cessation of 1,4-DCB exposure, recovery from the CNS and dermal effects was considered complete in some instances, but not in all cases. One case report of a death due to cardiac arrest was attributed to abuse of products containing 1,4-DCB (Alaufi et al., 2020; Maruthur et al., 2021).

6.1.2 Occupational Studies

Among a group of 58 workers who had worked 8 months to 25 years (average = 4.75 years) at a 1,4-DCB facility, repeated complaints of nasal and eye irritation were reported (Hollingsworth et al., 1956). Details of the eye and nasal irritation findings, which are characteristic of recurrent acute exposure, are presented in the Acute Exposure Section (Section 5.1). Numerous spot air samples of workroom atmospheres collected during several surveys of the facility showed concentrations of 1,4-DCB ranging from 5 to 725 ppm (30–4400 mg/m^3). TWA 8-hour exposure levels were not determined. All workers were occasionally given thorough examinations including measurement of blood hemoglobin, BUN, blood cell count, sedimentation rate and urinalysis. Blood tests and urinalysis did not reveal any indication of liver or kidney injury in the workers. Special attention was paid to the eyes of the employees since it was alleged at that time that 1,4-DCB may have caused cataracts in earlier nonindustrial clinical cases. Examination of the eyes did not detect pathological changes in the cornea or lens. The report did not state if exposure to other chemicals had occurred, although it was noted that the workers were not exposed to naphthalene.

Blood and urine samples were collected from 1,4-DCB workers in a Taiwanese insect repellent factory to look for markers of potential effects on hematological, liver, and kidney function (Hsiao et al., 2009). Participants included 46 workers and 29

administrative and medical workers with mean work durations of 11.8 and 9 years, respectively. Blood and urine samples were collected mid-workweek in the morning. Urine samples were also analyzed for free 2,5-DCP (non-conjugated metabolite). Statistically significant increased levels of 2,5-DCP ($p < 0.01$), white blood cell (WBC) count ($p < 0.01$), and alanine aminotransferase (ALT) ($p < 0.05$) were found in exposed workers compared to non-exposed workers, even after adjustment for confounding factors. WBC count and ALT was also significantly correlated to the concentration of urinary 2,5-DCP. When workers were stratified into onsite exposed (n = 33), onsite non-exposed (n = 13), and offsite non-exposed (n = 29), BUN and BUN/creatinine ratio was found to be significantly higher in onsite exposed workers ($p < 0.05$). The authors suggested that the increase in ALT in 1,4-DCB workers may indicate liver effects, although the increases in ALT and WBC count was considered minor, and the workers exhibited no obvious illness.

6.1.3 US Population Studies Using NHANES Biomonitoring Data

Several studies using NHANES data have found associations between various diseases or altered physiological states and the urinary 2,5-DCP concentration in survey participants. In general, dichlorophenols are suspected of having endocrine disrupting abilities (Rooney et al., 2019). However, due to the nature of these cross-sectional studies, causal relationships between 1,4-DCB exposure and associations with reported health conditions in NHANES participants are inherently difficult to establish. Limitations with using the survey data include a single urine sample, misclassification of self-reported data, and differences in 2,5-DCP levels that reflect differences in metabolism rather than differences in exposure.

Elliott et al. (2006) examined the relationship between pulmonary function and blood levels of VOCs in 953 adult participants (20–59 years old) from the third NHANES (1988–1994) study. Eleven VOCs including 1,4-DCB, were commonly identifiable in the blood. After adjustment for smoking, 1,4-DCB was the only VOC in which increased levels were significantly associated with reduced pulmonary function, including decreases in forced expiratory volume in one second (FEV_1) and maximum mid-expiratory flow rate (MMEFR) ($p < 0.05$, linear regression beta-coefficient). A significant inverse relationship was also found for 2,5-DCP in urine of a subgroup of the participants (n = 534) and FEV_1 and MMEFR. When the nontransformed values for 1,4-DCB were categorized into deciles, subjects in the highest decile of exposure had FEV_1 decrements of -153 ml (95% CI: -297 to -8, $p = 0.03$) and MMEFR decrements of -346 ml/sec (95% CI: -667 to -24, $p = 0.02$) compared to participants in the lowest decile.

A significant association between increasing interquartile levels of urinary 2,5-DCP and increasing prevalence of obesity ($p < 0.0001$, Cochran-Armitage trend test) was

observed in adults aged 20–85 years that participated in 2005–2008 NHANES studies (Wei et al., 2014). After adjusting for potential confounders, participants in the second, third and fourth interquartile groups had increased odds for obesity compared to participants in the lowest interquartile group ($p < 0.05$, multivariate logistic regression). A similar association was found between obesity and 2,5-DCP levels in children (See Section 6.2).

Following a similar methodology used by Wei et al. (2014), Wei and Zhu (2016a) observed a dose-dependent increase in the prevalence of diabetes among 3,063 adult NHANES 2007–2010 participants and their urinary 2,5-DCP level ($p < 0.0001$, Cochran-Armitage trend test). After adjusting for potential confounders, the highest interquartile group had increased odds for both diabetes and insulin resistance (characterized as type II diabetes) compared to participants in the lowest interquartile group.

The same research group also found a significant positive association ($p = 0.0025$, Cochran-Armitage trend test) across quartiles of urinary 2,5-DCP and metabolic syndrome in a subsample of non-diabetic adults (n = 1,706) participating in NHANES 2007–2010 cohorts (Wei and Zhu, 2016b). Metabolic syndrome comprises several health risk factors including increased waist circumference, elevated serum triglyceride, low high-density lipoprotein cholesterol, raised blood pressure and elevated blood glucose. Participants with at least three of the five risk factors were considered to have metabolic syndrome. After adjusting for potential confounders, the study found significantly increased odds for metabolic syndrome in participants in the third and fourth quartile compared to participants in the first quartile. Increased waist circumference and low high-density lipoprotein cholesterol showed the strongest association with urinary 2,5-DCP (ibid).

A larger sample size of NHANES 2003 – 2016 participants (n = 10,428) were examined by Cai et al. (2023) for associations between urinary 2,5-DCP and indicators of metabolic syndrome. A higher prevalence for metabolic syndrome was found to be positively associated with 2,5-DCP levels. After adjusting demographic, lifestyle, and dietary confounders, individuals in the highest versus lowest quartiles of 2,5-DCP concentrations had a 34% higher prevalence of metabolic syndrome. Higher urinary 2,5-DCP was also found to be associated with individual indicators of metabolic syndrome, including higher abdominal obesity, systolic blood pressure, waist circumference, and glycohemoglobin.

In further work by Zhu and Wei (2023), an inverse relationship was found between serum levels of the anti-aging hormone alpha-Klotho and urinary 2,5-DCP in a subsample of 1,485 adults aged 40–79 years in the 2013–2016 NHANES. With age- and sex-specific adjustment, the inverse association was strongest for older men

aged 60–79 years (p = 0.0008). No association was found for the middle age group (40–59 years) and for females. Klotho proteins play a protective role in aging and are essential components of endocrine fibroblast growth factors (FGF) receptor complexes, forming a unique endocrine system that regulates multiple metabolic processes in mammals. The FGF-Klotho endocrine axes may be involved in the pathogenesis of aging-related disorders, including diabetes, cardiovascular disease, cancer, chronic kidney disease, and neurological disorders.

Rooney et al. (2019) used the NHANES 2007–2010 data to examine associations between urinary 2,5-DCP in adults and higher prevalence of cancer, cardiovascular disease (CVD), lung disease, thyroid problems, and liver conditions. After stratifying increasing urinary 2,5-DCP levels into quartiles and adjusting for socioeconomic and lifestyle characteristics, higher urinary 2,5-DCP concentrations in the fourth quartile was significantly associated with greater prevalence of CVD (OR = 1.84, *p*-linear trend = 0.006) compared to the first quartile. Higher urinary 2,5-DCP concentrations in the fourth quartile were also associated with a greater prevalence of all cancers (OR = 1.50, *p*-linear trend = 0.05) combined, compared to the first quartile. The authors also noted that participants with higher 2,5-DCP concentrations tended to be obese. No statistically significant associations were found between urinary 2,5-DCP and lung diseases, thyroid problems, or liver conditions.

Associations between measures of kidney function and blood levels of six VOCs, including 1,4-DCB, were examined in 6070 adults participating in the 2003–2010 NHANES cohorts (Liu et al., 2022).

These authors also examined associations between 1,4-DCB concentration and vitamin D levels in blood. A significant inverse dose-response association was found between blood 1,4-DCB and Vitamin D as well as with estimated glomerular filtration rate (*p*-trend < 0.05). Vitamin D deficiency is common in, and may promote, the development and progression of chronic kidney disease.

6.2 Infants and Children

A significant association between increasing interquartile levels of urinary 2,5-DCP and increasing prevalence of obesity (*p* = 0.0001, Cochran-Armitage trend test) was observed in 6,770 children and adolescents aged 6–19 years that participated in 2005–2008 NHANES cohorts (Twum and Wei, 2011). After adjusting for potential confounders, children in the highest two quartiles had significantly increased odds for obesity compared to children in the lowest quartile group.

Wei and Zhu (2016c) also analyzed the association between urinary 2,5-DCP levels and data from thyroid function tests in 618 adolescents aged 12–18 selected from the 2007–2008 and 2011–2012 NHANES studies. Data collected on thyroid function

included free thyroxine levels (FT_4), free triiodothyronine levels (FT_3), thyroid stimulating hormone (TSH) levels and thyroglobulin (T_g) levels in serum. Hypothyroidism was defined by a TSH level above the normal range and either the FT_3 level or the FT_4 level below the normal range. When increasing urinary 2,5-DCP levels were stratified into quartiles, the prevalence of hypothyroidism in the first, second, third and fourth quartiles was, respectively, 3/156 (1.9%), 5/153 (3.3%), 6/157 (3.8%) and 2/164 (1.2%). The prevalence of hypothyroidism in children was stated to be 3.1%. The incidences in the second and third quartiles were not significantly greater than the incidence in first quartile. However, after adjusting for weighting and for possible confounders, increased odds for hypothyroidism was observed in the second, third and fourth quartiles compared to the first quartile.

6.3 Experimental Animals

This section includes summaries of both subchronic and chronic studies. A summary table (Table 10) is included at the end of the section.

Rats, rabbits, and guinea pigs were exposed by inhalation to 0, 96, 158, 341 or 798 ppm (0, 577, 950, 2050, or 4800 mg/m^3) 1,4-DCB for 7 or 8 hour/day, 5 days/week for up to 11 months (Hollingsworth et al., 1956). The rabbits and rats were from heterogenous stock raised in the lab, and guinea pigs were of a heterogeneous stock purchased from a commercial breeder. At the highest concentration, male (n = 19) and female (n = 15) rats were exposed up to 14 weeks, male (n = 16) and female (n = 7) guinea pigs were exposed for four weeks, and the male and female rabbits (n = 8 per sex) were exposed for up to 12 weeks. All animals were exposed 8 hours/day, with some sacrificed during the exposure period for histopathological analysis (number not specified). Tremors, weakness, eye irritation and unconsciousness were observed during the exposures, but were more likely acute/subacute toxic effects. Four rats, two guinea pigs, and four rabbits died during the exposures. Microscopic evaluation of organs at the end of the study found cloudy swelling and centrilobular necrosis in the liver of the animals, slight cloudy swelling of the tubular epithelium of the kidneys in female rats, and slight emphysema and congestion of the lungs in two rabbits.

In male rats (n = 20) and guinea pigs (n = 8 per sex) exposed to 341 ppm (2050 mg/m^3) 1,4-DCB for 6 months, the only histological finding was in some guinea pigs, in which cloudy swelling and focal necrosis in the liver was observed. In rats, guinea pigs, and rabbits exposed to 158 ppm (950 mg/m^3) for 8–11 months, cloudy swelling, or granular degeneration of centrilobular cells of “questionable significance” was seen only in the rats (Hollingsworth et al., 1956). Ten male mice and one female monkey were also exposed to this concentration, but no apparent toxic effects were found. No

signs of toxicity were noted in animals (i.e., 10 rats, 8 guinea pigs, 2 rabbits, 10 mice, and one female monkey) exposed to 96 ppm (580 mg/m^3) 1,4-DCB for 6–7 months.

In a chronic inhalation study by Riley et al. (1980), male and female SPF Wistar rats and female SPF Swiss mice were exposed to 0, 75, and 500 ppm (0, 451, and 3006 mg/m^3) 1,4-DCB for 5 hour/day, 5 days/week, for 76 weeks (rats) or 57 weeks (female mice). This study has not been peer-reviewed/published. In rats, only 5 animals/group/sex were examined at an interim kill (26–27 weeks) and at termination of exposure at 76 weeks. The remaining animals were exposed to clean air until study termination (27 to 34 animals/group/sex) at 109–112 weeks. Increased absolute and relative liver weights were observed at 1,4-DCB concentrations as low as 75 ppm in female rats at 26–27 weeks of exposure, and increased kidney and liver weights were observed in all 500 ppm exposure groups during either the interim sacrifice and/or the terminal exposure sacrifice at 76 weeks. Absolute and relative liver weights and absolute kidney weights were still elevated in 500 ppm female rats at 109–112 weeks. However, these findings were not accompanied by any related changes in clinical chemistry or histopathology. Nasal passages showed several lesions in the olfactory epithelium and nasal glands but since similar changes were also noted in the control groups, these changes were considered to be incidental or age related. The histopathology report showed an increased incidence of hepatocyte hyperplasia reported in 1,4-DCB-exposed female rats. Urinary and blood clinical chemistry found no relevant compound-related effects other than increased urinary protein and coproporphyrin excretion in 500 ppm rats.

The mouse study was reviewed from a secondary source (Loeser and Litchfield, 1983) because the primary mouse study report is not available. The mouse study was initiated with similar groups of male and female mice, but the male mice had to be terminated due to high mortality, likely due to respiratory infection. The background incidence of respiratory disease was high in all male and female groups. No exposure-related effects were observed in female mice, but the usefulness of this study is limited by the recurrent respiratory infections in the male mice as well as the unavailability of the original study report.

In an unpublished study sponsored by the Chemical Manufacturers Association Chlorobenzenes Program, the reproductive and developmental effects of inhaled 1,4-DCB over two generations were investigated in Sprague-Dawley rats (Tyl and Neeper-Bradley, 1989). Chronic toxicity in parental (F_0) and first generation (F_1) animals not directly related to reproduction or fetal developmental toxicity is reported here. The reproductive and developmental findings are reported in Section 7.2. Both generations of rats were exposed daily to mean 1,4-DCB analytical concentrations of 66, 211, and 538 ppm (398, 1,268, or 3,233 mg/m^3) for 6 hours/day. Male and female F_0 rats were exposed for 15 and 20 weeks, respectively. Male and female F_1 rats

were exposed for 21 and 22 weeks respectively. Female F_0 and F_1 rats were not exposed to 1,4-DCB during lactation days 1–4.

Reductions in body weight gain were observed during most of the 10-week pre-breed exposure period in 538 ppm F_0 and F_1 males, and during the first or second week of the study in the 211 ppm F_0 and F_1 males. Reduced body weight occurred occasionally in 538 ppm F_0 females during the 10-week pre-breed exposure period. During the breeding phase, maternal F_0 gestational body weight and weight gain were reduced at 538 ppm, and maternal F_0 body weight was also reduced on gestational day (GD) 20 at 211 ppm. F_1 adult females exhibited reduced gestational and lactational body weights at 538 ppm during the breeding phase.

Liver weights in the mid and high exposure groups in adult F_0 males were increased 16 and 38%, respectively, and were statistically significant ($p < 0.01$). All other F_0 and F_1 adult rats exposed to 538 ppm also exhibited increased liver weights. Other liver changes in adult rats at 211 ppm included increased liver to body weight ratios (F_0, F_1 males and F_0 females) and increased brain weight-to-liver weight ratios (F_0 males). Liver changes at 66 ppm were limited to a 5% increase in the liver-to-body weight ratios in F_0 males ($0.01 < p < 0.05$).

Treatment-related microscopic findings were limited to the liver and kidney. These included hyaline droplet nephrosis in all 1,4-DCB-exposed adult F_0 and F_1 male rats, and centrilobular hepatocellular hypertrophy in both the high dose male and female adult rats (Table 6). The increased incidence of nephrosis observed in F_1 males was comparable in type, severity and incidence to the nephrosis observed in the F_0 males at 211 and 538 ppm (1268 and 3233 mg/m^3). The study authors concluded that there was a No Observable Effect Level (NOEL) for the male rat hyaline droplet nephropathy, but this lesion is specific for male rats and not relevant to humans.

Table 6. Incidence of liver and kidney findings in F_0 and F_1 rats following chronic exposure to 1,4-DCB in the Tyl and Neeper-Bradley (1989) two-generation study[a].

Endpoint	Generation (sex)	0 ppm, (0 µg/m³)	66 ppm, (397 µg/m³)	211 ppm, (1268 µg/m³)	538 ppm, (3233 µg/m³)
Liver: hepatocellular hypertrophy	F_0 (male)	0/27	1/28	1/28	27/28**
	F_1 (male)	0/28	0/27	0/28	21/28**
	F_0 (female)	0/27	0/28	0/27	7/27**
	F_1 (female)	0/28	0/28	0/28	14/28**
Kidney: hyaline droplet nephrosis	F_0 (male)	11/27	27/28**	28/28**	28/28**
	F_1 (male)	10/28	27/27**	28/28**	28/28**
Kidney: tubular proteinosis	F_0 (male)	1/27	12/28**	11/28**	22/28**
	F_1 (male)	1/28	2/27	8/28*	15/28**
Kidney: granular cast formation	F_0 (male)	0/27	10/28**	15/28**	22/28**
	F_1 (male)	0/28	2/27	18/28**	16/28**
Kidney: interstitial nephritis	F_0 (male)	2/27	9/28*	14/28**	21/28**
	F_1 (male)	4/28	9/27	14/28**	25/28**
Kidney: interstitial fibrosis	F_0 (male)	0/27	6/28*	8/28**	5/28
	F_1 (male)	1/28	2/27	6/28	5/28
Kidney: tubular cell hyperplasia or hypertrophy	F_0 (male)	0/27	4/28	5/28	16/28**
	F_1 (male)	0/28	1/27	4/28	7/28*

[a] F_0 and F_1 male rats were exposed daily for approximately 15 and 21 weeks, respectively. F_0 and F_1 female rats were exposed for approximately 20 and 22 weeks, respectively, with the exception of lactation days 1–4.

* and ** – Statistically significant from control group at $p < 0.05$ and $p < 0.01$, respectively.

Abbreviations: F_0 – parent generation; F_1 – first generation; ppm – parts per million.

In a 13-week exposure study, groups of F344/DuCrj rats and Crj:BDF1 mice were exposed to 0, 25, 55, 120, 270 or 600 ppm (0, 150, 330, 720, 1420 or 3500 mg/m^3) 1,4-DCB for 6 hours/day, 5 days/week (Aiso et al., 2005a). In male rats, absolute and relative liver weights were increased beginning at 120 ppm. A consistent increase in absolute and relative liver weights in female rats began at 270 ppm. Absolute and relative kidney weights were increased in male rats beginning at 270 ppm and in female rats at 600 ppm. Absolute and relative spleen weights were increased in males at 600 ppm. The incidence of hepatic centrilobular hypertrophy was increased in males exposed to 270 and 600 ppm and in females exposed to 600 ppm. The incidence and severity of male rat renal hyaline droplets (positive for α-2μ-globulin), granular casts, tubular cell necrosis and cytoplasmic basophilia were increased at 270 and 600 ppm. The incidence of papillary mineralization in the renal pelvis was increased in the 600 ppm-exposed males. There were no histological changes in the kidneys of female rats. Hematological analysis in the males showed suggestive evidence for microcytic anemia due to decreases in hemoglobin beginning at 120 ppm, decreases in red blood cell count and hematocrit beginning at 270 ppm, and decreases in mean corpuscular volume and hemoglobin at 600 ppm (Table 7). Only hemoglobin was slightly decreased in 600 ppm females. The hematological effects in male rats were not accompanied with any anemia-related histopathological changes in the tissues. The authors therefore suggested that the hematological changes could be secondary to the male-rat specific α-2μ-globulin nephropathy, possibly related to effects on erythropoietin synthesis in the renal tubules.

Table 7. Key pathology and hematological effects in male and female rats exposed to 1,4-DCB for 13 weeks[a].

Endpoint	Sex	0 ppm, (0 µg/m^3)	25 ppm, (150 µg/m^3)	55 ppm, (330 µg/m^3)	120 ppm, (720 µg/m^3)	270 ppm, (1420 µg/m^3)	600 ppm, (3500 µg/m^3)
Liver: centrilobular hypertrophy	Male	0/10	0/10	0/10	0/10	3/10	9/10††
Kidney: hyaline droplets[b]	Male	0/10	1/10	0/10	0/10	10/10††	9/10††
Kidney: tubular cell necrosis	Male	0/10	0/10	0/10	0/10	10/10††	10/10††
Kidney: papilla mineralization	Male	0/10	0/10	0/10	0/10	1/10	7/10††
RBCs (10^6/µl)	Male	9.35 ± 0.12	9.31 ± 0.19	9.37 ± 0.17	9.16* ± 0.15	8.86** ± 0.16	8.68** ± 0.18
Hemoglobin (g/dl)	Male	16.1 ± 0.2	16.0 ± 0.4	16.1 ± 0.2	15.7** ± 0.3	15.3** ± 0.2	14.6** ± 0.3
Hematocrit (%)	Male	47.3 ± 0.7	47.0 ± 1.4	47.3 ± 0.9	46.1 ± 0.9	44.8** ± 0.7	43.0** ± 1.0
MCV (fl)	Male	50.5 ± 0.5	50.5 ± 0.7	50.5 ± 0.6	50.3 ± 0.4	50.6 ± 0.3	49.5** ± 0.6
MCH (pg)	Male	17.3 ± 0.3	17.2 ± 0.2	17.3 ± 0.3	17.1 ± 0.2	17.3 ± 0.3	16.8** ± 0.1
Liver: centrilobular hypertrophy	Female	0/10	0/10	0/10	0/10	0/10	3/10
Hemoglobin (g/dl)	Female	15.9 ± 0.5	16.2 ± 0.3	15.7 ± 0.3	15.8 ± 0.4	16.0 ± 0.3	15.3* ± 0.6

(a) Pathology findings presented as number affected / number examined; hematology data are means ± standard deviations.
(b) Moderate, marked, and severe grades combined.
† and †† Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively, by Chi square test.
* and ** Significantly different from control at $p < 0.05$ and $p < 0.01$, respectively, by Dunnett's test.
Abbreviations: fl – femtoliters; g/dl – grams per deciliter; MCH – mean corpuscular hemoglobin; MCV – mean corpuscular volume; 10^6/µl – million cells per microliter; pg – picograms; RBC – red blood cell count.

Blood biochemistry revealed increased total cholesterol and phospholipid in 270 and 600 ppm males and 600 ppm females. Total protein and albumin were increased in all 600 ppm rats. BUN and creatinine were increased in the 600 ppm males, indicative of decreased glomerular filtration resulting from kidney damage. No signs of toxicity were seen in the respiratory tract of mice or rats exposed to 1,4-DCB.

In the 13-week exposure study in male and female mice by Aiso et al. (2005a), absolute and relative liver weights were increased in females beginning at 270 ppm. In males, absolute liver weight was increased at 600 ppm and relative liver weight was increased in all exposed groups. Absolute kidney weight was increased in 600 ppm females and relative kidney weight was increased in 270 and 600 ppm males. An increased incidence and severity of centrilobular hypertrophy of hepatocytes were observed in males at 270 and 600 ppm and in the females at 600 ppm. Focal liver necrosis was observed in some 600 ppm-exposed males. Blood biochemistry revealed increased aspartate aminotransferase (AST) in 600 ppm males and increased ALT in 270 and 600 ppm males and 600 ppm females. Total cholesterol and protein were increased in 600 ppm males and females, while BUN was increased only in 600 ppm males. There were no histological changes in the kidneys of mice of either sex.

In a two-year inhalation study, groups of F344/DuCrj rats and Crj:BDF1 mice (50 animals/sex/dose for each rodent species) were exposed to 0, 20, 75 or 300 ppm (0, 120, 450 or 1800 mg/m^3) 1,4-DCB for 6 hour/day, 5 days/week (Aiso et al., 2005b). Liver, kidney, and nasal epithelium were the primary targets of chronically inhaled 1,4-DCB in rodents. In rats, significantly decreased survival of 300 ppm males was observed, and was attributed to chronic progressive nephropathy (CPN), leukemia or other tumors (survival, log-rank test: 33/50, 34/50, 29/50, and 18/50 for 0, 20, 75, and 300 ppm groups, respectively). Specifically regarding CPN deaths, 6 and 11 male rats died from this disease in the control and 300 ppm groups, respectively. Increases in absolute and relative liver weights were observed in male and female rats exposed to 300 ppm and in kidneys of males exposed to 300 ppm. Including the control groups, CPN was observed in nearly all male rats (49 or 50 cases per exposure group), and most female rats (43 to 48 cases per exposure group), but the incidence and severity of CPN did not exhibit a statistically significant trend with increasing 1,4-DCB exposure. However, the overall severity of CPN, a spontaneous disease, was greater in male rats compared to female rats. Unlike their 13-week study in rodents (Aiso et al., 2005a), excessive accumulation of α-2μ-globulin was not found in any of the male rat groups exposed to 1,4-DCB for 2 years.

The principal pathology findings for noncancer effects in rats, other than CPN, are shown in Table 8. Histopathological examination revealed an increased incidence of centrilobular hypertrophy of hepatocytes and an increased incidence of papillary

mineralization and hyperplasia of the pelvic urothelium in the kidneys in 300 ppm males. In the nasal cavity of female rats, there was an increased severity of eosinophilic globules at 75 and 300 ppm, and an increased incidence of the same lesion in the respiratory epithelium at 300 ppm. The increase in eosinophilic globules was closely related to a marked decrease in the number of olfactory cells in the olfactory epithelium at 300 ppm. The incidence of respiratory metaplasia of the nasal gland epithelium was also increased in the females at 300 ppm. A statistically significant ($p < 0.0001$) exposure-response relationship was observed for many of the endpoints listed in Table 8.

Table 8. Principal noncancer pathology findings in the 2-year 1,4-DCB inhalation study in rats (Aiso et al. (2005b)).

Endpoint	Sex	0 ppm [a], (0 µg/m^3)	20 ppm, (120 µg/m^3)	75 ppm, (450 µg/m^3)	300 ppm, (1800 µg/m^3)
Kidney: papilla mineralization [c]	Male	0/50†	1/50	0/50	41/50**
Kidney: pelvic urothelial hyperplasia [c]	Male	7/50†	8/50	13/50	32/50**
Liver: hepatocellular centrilobular hypertrophy [c]	Male	0/50†	0/50	0/50	5/50*
Nasal epithelium: olfactory eosinophilic globules – slight	Female	22/50	17/50	7/50	3/50
Nasal epithelium: olfactory eosinophilic globules – moderate	Female	21/50	27/50	16/50	27/50
Nasal epithelium: olfactory eosinophilic globules – marked	Female	6/50†	2/50	23/50**	20/50**
Nasal epithelium: olfactory eosinophilic globules – moderate and marked combined [b]	Female	27/50†	29/50	39/50*	47/50**
Nasal epithelium: respiratory eosinophilic globules [c]	Female	11/50†	10/50	14/50	38/50**
Nasal epithelium: respiratory metaplasia: nasal gland [c]	Female	5/50†	4/50	4/50	33/50**

* and ** - Statistically significant from control group at $p \leq 0.05$ and $p \leq 0.01$, respectively, by Chi-square test as calculated by the authors

(a) Statistical notation in control column, † $p \leq 0.05$, indicates significant positive trend for endpoint by Cochran-Armitage test, conducted by OEHHA

(b) Fisher exact test for combined moderate and marked olfactory eosinophilic globules conducted by OEHHA - * $p \leq 0.05$ and ** $p \leq 0.01$, two-tailed.

(c) Slight and moderate pathologic grades of severity for these lesions are combined.

Although the presence of eosinophilic globules is a spontaneous lesion in aged male and female rats, there was an increased incidence of the severity (marked) of this lesion in female rats exposed to 75 ppm.

This two-year 1,4-DCB exposure study was previously presented in an unpublished summary report by the Japan Bioassay Research Center (JBRC, 1995), which includes additional information not described in the peer-reviewed published study by Aiso et al. (2005b). In this report, blood biochemistry results noted significantly increased total cholesterol, phospholipid, BUN, creatinine, and calcium in the male 300 ppm rats compared to the control group. In 20 ppm and 300 ppm female rats, total protein was significantly reduced, and total bilirubin, BUN, and potassium were significantly increased compared to the control group. Values for the blood chemistry results were not provided. The report also notes that no clinical signs of toxicity were observed in any of the exposed rats throughout the exposure period.

In the two-year mouse study, a decreased survival rate was observed in 300 ppm males, attributed to an increase in the number of liver tumor deaths (Aiso et al., 2005b). Clinical signs of toxicity were not observed in any of the exposed mice. Decreased body weight was also observed in the last 15–20 weeks of exposure in 300 ppm males and was 12% less than controls at the end of two years. Absolute and relative liver weights were increased in both males and female mice at 300 ppm. Absolute and relative kidney weights were increased in 300 ppm-females and relative kidney weight was increased in 300 ppm-males.

The principal pathology findings of the noncancer effects in mice are also shown in Table 9. Increased incidence of centrilobular hypertrophy of hepatocytes occurred in 300 ppm males, but no histopathological evidence of hepatocellular injury was observed in any of the 1,4-DCB-exposed groups of mice of either sex. Respiratory metaplasia was significantly increased in 75 ppm males in both the nasal gland epithelium and the nasal olfactory epithelium, but neither lesion was significantly increased over control values in the 300 ppm males. Significantly increased respiratory metaplasia of the nasal olfactory epithelium was observed in 300 ppm females. No significant increase in severity grade with increasing exposure concentration was observed for the nasal lesions in mice.

Table 9. Principal noncancer pathology findings in the 2-year 1,4-DCB inhalation study in mice (Aiso et al. 2005b).

Endpoint	Sex	0 ppm[a], (0 µg/m³)	20 ppm, (120 µg/m³)	75 ppm, (450 µg/m³)	300 ppm, (1800 µg/m³)
Respiratory metaplasia: nasal gland[b]	Male	37/49	42/49	47/50*	41/49
Respiratory metaplasia: olfactory epithelium[c]	Male	23/49	30/49	38/49**	24/49
Liver: hepatocellular centrilobular hypertrophy[c]	Male	0/49†	0/49	0/50	34/49**
Respiratory metaplasia: olfactory epithelium[d]	Female	7/50†	6/50	2/49	20/50**

* and ** - Statistically significant from control group at $p \leq 0.05$ and $p \leq 0.01$, respectively, by Chi-square test as calculated by the authors

(a) Statistical notation in control column, † $p \leq 0.05$, indicates significant positive trend for endpoint by Cochran-Armitage test, conducted by OEHHA

(b) Slight, moderate, and marked severity grades combined

(c) Slight and moderate severity grades combined

(d) Slight severity grade only in all exposure groups

The original summary report by JBRC (1995) for this two-year inhalation study in mice also shows a significant ($p < 0.05$) increase in mineralization of the testis in males in the 75 and 300 ppm groups (27/49, 35/49, 42/50, and 41/49 in the 0, 20, 75, and 300 ppm groups, respectively; Cochran-Armitage test for trend: p = 0.0061), but the importance of this finding was not discussed. This lesion was not reported or discussed in the peer reviewed publication of the same study (Aiso et al., 2005b). Blood chemistry results presented only in JBRC (1995) states that total cholesterol,

glutamic oxaloacetic transaminase (also known as aspartate aminotransferase, or AST), ALT, LDH and ALP activity were significantly increased in both 300 ppm males and females compared to their respective control groups. In addition, total protein, albumin, total bilirubin, BUN, and calcium were significantly greater in 300 ppm females compared to the control group. Values for the blood chemistry results were not provided.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals

Reference	Animal model and exposure	Results	Point of departure
Hollingsworth et al. (1956)	Groups of rats and guinea pigs exposed to 96, 158, 341, 798 ppm for 7–8 hours/day, 5 days/week for up to 6–11 months Rabbits exposed to 96, 158, 798 ppm for 7–8 hours/day, 5 days/week for up to 6–11 months Groups of mice exposed to 96 or 158 ppm for 7 hours/day, 5 days/week for up to 6–11 months One monkey each exposed to 96 or 158 ppm for 7 hours/day, 5 days/week for 6–11 months	In rats, liver toxicity observed at 798 ppm, and possible liver toxicity at 158 ppm in animals exposed for up to 11 months. Kidney toxicity observed in female rats only at 798 ppm. In guinea pigs, liver toxicity observed at 341 ppm and above. In rabbits, liver and pulmonary toxicity observed at 798 ppm No toxic findings in mice and monkeys	NOAEL: In rats, 341 ppm or 158 ppm Guinea pigs, rabbits, mice, and monkeys: 158 ppm LOAEL: In rats, 158 or 798 ppm In guinea pigs and rabbits, 158 ppm In mice and monkeys, NA

Abbreviations: ppm – parts per million; NA – not applicable.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals (continued)

Reference	Animal model and exposure	Results	Point of departure
Riley et al. (1980)	Male and female Wistar rats and female SPF Swiss mice (76–79/sex/dose) exposed 5 hours/day, 5 days/week for 76 weeks (rats) or 57 weeks (mice)	Liver hypertrophy and ↑ kidney weight observed at 500 ppm mainly in female rats, but no accompanying liver or kidney toxicity. No increase in nasal lesions compared to controls. ↑ urinary protein and coproporphyrin excretion at 500 ppm Female mice data compromised by respiratory infection	NOAEL: 500 ppm LOAEL: NA
Tyl and Neeper-Bradley (1989)	Male and female Sprague-Dawley rats (28 per sex) exposed to 0, 66, 211 or 538 ppm for 6 hours/day, 7 days/week for 15 weeks in F_0 males and 20 weeks in F_0 females covering pre-mating, mating, and gestation-lactation (females only) phases. Similar protocol used for F_1 rats although total exposures were 21–22 weeks	↓ BW in F_0 and F_1 males and females at 538 ppm during part or most of exposure ↓ BW in F_0 females at 211 ppm on GD 20. ↑ absolute and relative liver weight at 211 and 538 ppm in one or both generations of male and females. ↑ hepatocellular hypertrophy in F_0 and F_1 male and females at 538 ppm ↑ hyaline droplet nephrosis in all treated F_0 and F_1 males	NOAEL: NA LOAEL: 66 ppm for hyaline droplet nephrosis in male rats

Abbreviations: ↓ – decreased significantly ($p < 0.05$) relative to control; ↑ – increased significantly ($p < 0.05$) relative to control; BW – body weight; F_1 – first offspring generation; F_0 – parental generation; GD – gestation day; LOAEL – Lowest Observed Adverse Effect Level; NA – not applicable; NOAEL – No Observed Adverse Effect Level; ppm – parts per million.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals (continued)

Reference	Animal model and exposure	Results	Point of departure
Aiso et al. (2005a)	Male and female F344 rats exposed to 0, 25, 55, 120, 270, 600 ppm for 6 hours/day, 5 days/week for 13 weeks n = 10 rats per sex per dose	↑ absolute and relative liver weight in males at 120 ppm and above, and in females at 270 ppm and above ↑ absolute and relative kidney weights in males at 270 ppm and above, and in females at 600 ppm ↑ absolute and relative spleen weights in males at 600 ppm ↑ hepatocellular hypertrophy at 270 ppm and above in males, and at 600 ppm in females ↑ hyaline droplet nephrosis in males 270 ppm and above ↑ evidence of microcytic anemia in males beginning at 120 ppm and above ↑ BUN and creatinine in males at 600 ppm	NOAEL: 55 ppm LOAEL: 120 ppm for evidence of microcytic anemia in males probably secondary to α2μ globulin nephropathy

Abbreviations: ↑ – increased significantly ($p < 0.05$) relative to control; BUN – blood urea nitrogen; LOAEL – Lowest Observed Adverse Effect Level; n – number; NOAEL – No Observed Adverse Effect Level; ppm – parts per million.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals (continued)

Reference	Animal model and exposure	Results	Point of departure
Aiso et al. (2005a; continued)	Male and female BDF1 mice exposed to 0, 25, 55, 120, 270, 600 ppm for 6 hours/day, 5 days/week for 13 weeks n = 10 mice per sex per dose	↑ absolute liver weight at 600 ppm in males and 270 ppm and above in females ↑ relative liver weight in males at 25 ppm and above, and in females at 270 ppm and above ↑ absolute weights in females at 600 ppm ↑ hepatocellular hypertrophy at 270 ppm and above in males, with some focal liver necrosis at 600 ppm ↑ hepatocellular hypertrophy in females at 600 ppm ↑ AST at 600 ppm and ALT at 270 ppm and above in males ↑ ALT in females at 600 ppm ↑ BUN in males at 600 ppm, and ↑ cholesterol and protein in 600 ppm males and females	NOAEL: 270 ppm LOAEL: 600 ppm for focal liver necrosis in males

Abbreviations: ALT – alanine aminotransferase; ↑ – increased significantly ($p < 0.05$) relative to control; AST – aspartate aminotransferase; BUN – blood urea nitrogen; BW – body weight; LOAEL – Lowest Observed Adverse Effect Level; n – number; NOAEL – No Observed Adverse Effect Level; ppm – parts per million.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals (continued)

Reference	Animal model and exposure	Results	Point of departure
Aiso et al. (2005b)	Male and female F344 rats exposed to 0, 20, 75, 300 ppm for 6 hours/day, 5 days/week for 2 years n = 50 rats per sex per dose	↓ survival in males at 300 ppm ↑ absolute and relative liver weight at 300 ppm in males and females ↑ hepatocellular hypertrophy in males at 300 ppm ↑ absolute and relative kidney weight at 300 ppm in males ↑ kidney papillary mineralization and hyperplasia in males at 300 ppm ↑ incidence of marked nasal olfactory eosinophilic globules in females at 75 ppm ↑ incidence of nasal respiratory eosinophilic globules and metaplasia in females at 300 ppm	NOAEL: 20 ppm LOAEL: 75 ppm for increased severity of nasal lesions in females

Abbreviations: ↓ – decreased significantly ($p < 0.05$) relative to control; ↑ – increased significantly ($p < 0.05$) relative to control; LOAEL – Lowest Observed Adverse Effect Level; n – number; NOAEL – No Observed Adverse Effect Level; ppm – parts per million.

Table 10. Summary of subchronic and chronic effects of 1,4-DCB inhalation exposure in experimental animals (continued)

Reference	Animal model and exposure	Results	Point of departure
Aiso et al. (2005b; continued)	Male and female BDF1 mice exposed to 0, 20, 75, 300 ppm for 6 hours/day, 5 days/week for 2 years n = 50 mice per sex per dose	↓ survival and body weight in males at 300 ppm ↑ absolute and relative liver weight at 300 ppm in males and females ↑ absolute and relative kidney weight at 300 ppm in females, relative kidney weight ↑ in 300 ppm males ↑ hepatocellular hypertrophy in males at 300 ppm ↑ nasal olfactory metaplasia in females at 300 ppm	NOAEL: 75 ppm LOAEL: 300 ppm for increased incidence of nasal lesions in females

Abbreviations: ↓ – decreased significantly ($p < 0.05$) relative to control; ↑ – increased significantly ($p < 0.05$) relative to control; LOAEL – Lowest Observed Adverse Effect Level; n – number; NOAEL – No Observed Adverse Effect Level; ppm – parts per million.

7. Developmental and Reproductive Toxicity

7.1 Human Developmental and Reproductive Toxicity

Summarized below are case reports of 1,4-DCB exposure during pregnancy that resulted in injury to the mother. In addition, several biomonitoring studies are summarized in which associations were found between urinary levels of 2,5-DCP and altered developmental endpoints and milestones in infants in children. No studies were found for developmental and reproductive effects in humans with quantifiable inhalation exposures to 1,4-DCB.

A pregnant woman who ingested toilet air-freshener blocks containing mainly 1,4-dichlorobenzene (1 to 2 blocks per week) throughout her pregnancy did not show any abnormalities in the infant (Campbell and Davidson, 1970). The mother showed signs

of hemolytic anemia when admitted but was reversible after cessation of exposure. There was no reported jaundice or presence of methemoglobin in serum, and liver function tests and urinalysis were normal.

In an abstract for a case report, a 28-year-old pregnant woman with a history of chronic ingestion of 1,4-DCB and schizoaffective disorder was admitted to the hospital in labor at 36-weeks of gestation (Vigh et al., 2019). She self-reported daily ingestion of approximately 1–4 mothballs over fourteen years and admitted to cessation of ingestion only after the discovery of pregnancy at 16 weeks of gestation. She showed signs of tremor, ataxia, and ichthyosis-like dermatosis. The baby was delivered by caesarian section with a body weight of 2,325 grams (23rd percentile). Placental weight was 370 grams (<3rd percentile for gestational age). The female newborn exhibited transient hypoglycemia, periodic lip-smacking and facial twitching. These symptoms resolved within 48 hours. An MRI of the mother revealed degenerative leukoencephalopathy whereas none was seen in the baby. 2,5-DCP was detected in both mother and baby's urine suggesting placental transmission of the metabolite. 1,4-DCB was also detected in the mother's blood at 24 µg/ml (normal range listed by the authors was <2 µg/ml).

Wolff et al. (2008) measured prenatal exposures to phthalates and phenols expected to be hormonally active and that could potentially alter fetal development. As part of this assessment, urinary 2,5-DCP was measured in a cohort of 404 healthy multiethnic women in New York City during their third trimester of pregnancy and the size of infants at birth was recorded. The authors found higher urinary levels of 2,5-DCP predicted lower birth weight in male infants. The mean birth weight of male infants in the third tertile for urinary 2,5-DCP concentration was 210 g less than when compared to male infants in the first tertile (p = 0.0016, 95% CI: -348, -71). Birth weight-predicted means in the study were adjusted for race/ethnicity, gestational age, creatinine (natural log transformed), smoking during pregnancy, maternal education, marital status, and pre-pregnancy body mass index (BMI) and were limited to samples with ≥ 20 mg/dL creatinine. The authors noted that this 200 gram deficit in birth weight is comparable to the reduction in birth weight seen in active smoking during pregnancy.

Relationships between male newborn body size and prenatal exposure to phthalates and phenols were also investigated in a French study by Phillipat et al. (2012). Maternal urinary samples were collected between 6 and 30 weeks of gestation and analyzed for chemical metabolites, including 2,5-DCP (n = 191 pregnant women). Birth weight decreased by 49 g (95% CI: -86, -13) in association with a 1-unit increase in natural log transformed 2,5-DCP concentration. After stratifying into tertiles, boys in the highest exposure tertile were significantly lighter by 152 g compared to boys in the lowest tertile (p-trend = 0.03, 95% CI: -299, -5). Adjusting for

many potential confounders did not alter the association. No association was found between prenatal urinary 2,5-DCP and change in birth length or change in head circumference. The authors suggested that the greater decrease in body weight in the third tertile found in the Wolff et al. (2008) study may have been a result of higher prenatal 2,5-DCP concentration (Wolff et al. median 2,5-DCP concentration – 53 µg/L; Phillipat et al. median 2,5-DCP concentration – 6.4 µg/L).

Age of menarche and exposure to endocrine-disrupting chemicals was investigated in female participants 12–17 years of age (n = 440) that had completed the reproductive health questionnaire and laboratory examination portion of the 2003–2008 NHANES (Buttke et al., 2012). The weighted survival analysis model, adjusted for race/ethnicity and BMI, found a significant inverse association of urinary 2,5-DCP with age of menarche (hazard ratio = 1.10; 95% CI: 1.01, 1.19; $p < 0.025$). Exposure to other potential endocrine-disrupting agents (total parabens, bisphenol A, triclosan, benzo[henone-3, total phthalates, and 2,4-DCP) were not significantly associated with age of menarche.

In the Breast Cancer and Environment Research Program (BCERP) study, Wolff et al. (2015) investigated associations between urinary concentrations of 2,5-DCP and other phenolic chemicals in girls and pubertal onset of breast development (thelarche) and pubic hair (pubarche). Girls ages 6–8 at the beginning of the study were followed for 7 years. Higher concentrations of urinary 2,5-DCP in the fifth quintile was significantly associated with younger age of thelarche (9 months earlier) compared to the first quintile. Urinary 2,5-DCP was also associated with earlier age at pubarche (approximately 25% increased risk for the fifth versus first quintile). Stronger associations of phenols with thelarche were found among younger, heavier girls.

Wolff et al. (2017) also investigated associations with age at menarche in the BCERP study. Girls (n = 1051) 6–8 years of age at the beginning of the study were followed for up to 11 years. Higher urinary 2,5-DCP was significantly associated with earlier menarche; Kruskal-Wallace test of 2,5-DCP biomarker median differed across three menarche age groups ($p < 0.05$). The 2,5-DCP effect on menarche was the same regardless of BMI. When comparing girls in the fifth and first quintile concentrations of 2,5-DCP, adjusted median age for menarche was 7 months earlier for 2,5-DCP. The authors noted that since early puberty is believed to be a risk factor for metabolic disease and breast cancer, hormonal effect of environmental agents during puberty may be an indirect pathway for disease later in life.

Harley et al. (2019) conducted a longitudinal study that investigated in utero and peripubertal exposures of phthalates, parabens, and phenols in mostly Latina pregnant women and their children (338 children) from Salinas Valley, California.

Mothers were interviewed at two points during their pregnancy at which time spot urine samples were collected. One urine sample were collected from their children at 9 years of age. Pubertal timing was assessed among 179 girls and 159 boys every 9 months between ages 9 and 13. A significant association ($p < 0.05$) was observed for later pubarche in girls with a 2-fold increase in peripubertal 2,5-DCP concentration (mean shift = 1.0 month, 95% CI: 0.1, 1.9). No association was observed in girls for age at thelarche or menarche and peripubertal 2,5-DCP concentration. In addition, no significant association was found between prenatal urinary 2,5-DCP concentration and age of pubertal milestones in girls (i.e., thelarche, pubarche and menarche). In boys, no association was found between prenatal or peripubertal 2,5-DCP and pubertal milestones (gonadarche and pubarche).

The results of the Harley et al. (2019) study contrasts with the results of the NHANES study of Buttke et al. (2012), in which urinary 2,5-DCP concentrations in girls were associated with earlier menarche. The results also contrast with the BCERP findings (Wolff et al., 2015; Wolff et al., 2017) in which 2,5-DCP in girls was associated with earlier thelarche, pubarche and menarche. Harley et al. (2019) suggested that timing of exposure assessment may be a factor in these discrepancies with other studies.

Buckley et al. (2018) assessed associations of prenatal environmental phenol biomarkers with respiratory and allergic outcomes among school-aged children (age 6–7 years, n = 159) participating in a prospective pregnancy cohort study (Mount Sinai Children's Environmental Health Study) in New York City. This study demonstrated associations of third trimester maternal urinary 2,5-DCP concentrations with increased odds of ever being diagnosed with asthma (OR: 1.51, 95% CI: 0.93, 2.46), emergency room visits for an asthma attack in the past 12 months (OR: 2.07, 95% CI: 1.17, 3.68), and rashes, eczema, or hives in the past 12 months (OR: 1.71, 95% CI: 1.15, 2.55). These outcomes were statistically significant in boys, but no positive associations were seen when compared with girls (Buckley et al., 2018). The authors suggested 1,4-DCB and other phenol chemicals may induce immunologic changes leading to adverse respiratory and allergic outcomes. In particular, the role of estrogen in immune response suggests the potential for endocrine disrupting chemicals to influence the development of asthma and allergic disease.

7.2 Reproductive and Developmental Studies in Animals

Developmental toxicity studies in animals

In an unpublished study sponsored by the Chlorobenzene Producers Association, groups of pregnant SPF strain Alderley-Park rats (20–24 per group) were exposed whole-body to 1,4-DCB air concentrations of 0, 75, 200 and 500 ppm (0, 451, 1202, and 3005 mg/m^3) for 6 hours/day from GD 6 to 15. This study was conducted by Hodge et al. (1977), but the original study could not be obtained by OEHHA. However, it was summarized and evaluated by the United States Environmental Protection Agency (US EPA, 1989) and is presented here. The dams were sacrificed on GD 21 with subsequent examination of fetuses and maternal tissues. Half of the fetuses from each litter were examined for visceral malformations, and the other half prepared and examined for skeletal malformations and degree of ossification. Maternal body weight and body weight gain was unaffected by 1,4-DCB exposure. Additionally, no treatment-related macroscopic organ tissue lesions or histological changes of lung and liver were observed. 1,4-DCB exposure did not adversely affect the number of implantations, resorptions, viable fetuses, corpora lutea, or sex ratios. In addition, no developmental effects including fetal weight, litter weight, external abnormalities, and skeletal and visceral abnormalities were found. Since there were no differences in maternal clinical signs of any treatment group and no differences in other fetal alterations and anomalies, the high dose tested in this study was not high enough to be considered as the maximum tolerated dose.

A developmental study by Hayes et al. (1985) exposed artificially inseminated New Zealand White (NZW) rabbits whole-body to 1,4-DCB at air concentrations of 0, 100, 300, or 800 ppm (0, 601, 1803 or 4808 mg/m^3), 6 hours/day on GD 6–18. A significant decrease in maternal body weight gain during the first 3 days of exposure was seen in the 800-ppm group (Table 11). However, the maternal weight gain was not significantly reduced at later time periods in the study. Overall, rabbits in the 800-ppm group gained less weight than the controls (28 g gain versus 185 g in the controls) during GD 6–18, but this weight change was not statistically significant. Following cessation of 1,4-DCB exposure, the 800 ppm group gained significantly more weight than controls during GD 19–28.

At sacrifice on GD 29, no differences between treated and control groups in the mean number of corpora lutea per dam, the mean number of implantation sites per dam, the mean number of resorptions per litter, or the number of totally resorbed litters were found (Hayes et al., 1985). An additional observation presented in the industry study report (Hayes et al., 1982), but not in the published study by Hayes et al. (1985), noted that there were no dead fetuses found in any of the exposure groups. Absolute and relative weight of the kidney and liver in the does were unaffected by

1,4-DCB exposure. At 300 ppm, there was a significant increase ($p \leq 0.05$; modified Wilcoxon test) in the percentage of resorbed implantations (16% versus 7% in the controls) and in the number of litters with resorptions (63% versus 29% in the controls) (Table 11). However, the incidence of resorptions in the 100 and 800 ppm groups were not different from control. The percentage of litters with resorptions in the 300 ppm group were within the range reported for historical controls (Historical mean% of litters with resorptions: 40%, range 0% to 70%, 22 study control groups). The study authors concluded that the increased percentages of resorbed implantations and litters with resorptions at 300 ppm were not chemical- or dose-related.

In the fetuses, no treatment-related change in body weight and crown-rump length was observed. The incidence of major malformations in 1,4-DCB-exposed groups, both singly and in total, was not different from control. A significant increase ($p \leq 0.05$; modified Wilcoxon test) in the incidence of retroesophageal right subclavian artery was observed in the 800 ppm offspring on a fetal and litter basis (five 800 ppm litters versus one in controls; 18% of 800 ppm group litters affected and 5% (6/119) of total fetuses examined) (Table 11). The authors considered this fetal effect to be a normal, minor variation of the circulatory system that had been observed in 2% of the control animals in their laboratory (range and number of control fetuses examined not provided). However, in its review, US EPA (1989) remarked that this alteration probably represents a developmental effect.

Table 11. Summary of main maternal and fetal findings for the inhalation developmental study in rabbits exposed to 1,4-DCB (Hayes et al., 1985).

Endpoint	0 ppm (0 µg/m³)	100 ppm (601 µg/m³)	300 ppm (1803 µg/m³)	800 ppm (4808 µg/m³)
Number of dams	28	24	24	28
Maternal BW gain – GD 6–18	8 ± 68[a]	2 ± 104	-32 ± 165	-82 ± 122*
Maternal BW gain – GD 9–11	64 ± 137	64 ± 46	42 ± 85	39 ± 117
Maternal BW gain – GD 12–14	56 ± 89	78 ± 68	84 ± 73	65 ± 99
Maternal BW gain – GD 15–18	57 ± 52	44 ± 83	39 ± 75	6 ± 146
Maternal BW gain – GD 19–28	63 ± 136	97 ± 246	126 ± 192	189 ± 118*
Maternal BW gain – GD 6–28	248 ± 165	286 ± 274	259 ± 288	217 ± 204
% implantations resorbed (fetal incidence / total fetuses)	7 (15/225)	10 (19/195)	16 (33/208)[b]	6 (15/233)
% litters with resorptions (litter incidence / total litters)	29 (8/28)	54 (13/24)	63 (15/24)[b]	39 (11/28)
No. of fetuses examined (litters)	210 (28)	176 (23)	175 (22)	218 (28)
Fetal visceral examination (no.)	115	94	93	119
Fetal skeletal examination (no.)	210	176	175	218
Fetal body weight (g)	37.94 ± 6.56[a]	37.06 + 7.48	38.57 ± 5.59	37.01 ± 4.39
Total no. of fetuses with retroesophageal right subclavian artery (total litters)	1 (1)	0 (0)	1 (1)	6†,(5)†
Total no. of fetuses with major malformations (total litters)	8 (7)	6 (4)	3 (3)	11 (7)

(a) Mean ± standard deviation

* Significantly different from control value ($p < 0.05$) by Dunnett's test.

†Significantly different from control ($p < 0.05$) by a modified Wilcoxon test.

Abbreviations: BW – body weight; g – grams; GD – gestation day

In humans, a retroesophageal right subclavian artery is one of the most common aortic arch anomalies (Crary and Fox, 1978; Ocaya, 2015). This malformation is usually without clinical symptoms, but in some cases may cause compression of the

esophagus or the trachea, or both, possibly leading to swallowing or breathing difficulties. There is also a higher risk of clot-related events and aneurysm.

In an examination of control data for embryo-fetal developmental effects in NZW rabbits, Paradis et al. (2019) reported the incidence of retroesophageal [right] subclavian artery was 0.14% in the fetuses (7 of 4949 fetuses) and 0.94% among the litters (5 of 532 litters). Similar control incidences for this blood vessel anomaly (termed aberrant right subclavian artery by the authors) in NZW rabbits was observed in a Japanese lab: 0.12% (range: 0 – 1.67%, n = 3803 fetuses) from 1994 to 2000, and 0.05% (range: 0%–0.65%, n = 5580) from 2000 to 2010 (Ema et al., 2012). Development of the heart region in rabbit fetuses, when anomalies such as a retroesophageal right subclavian artery would arise, occurs during GD 12–15.

In an unpublished study sponsored by the Chemical Manufacturers Association Chlorobenzenes Program, the effects of inhaled 1,4-DCB on parental fertility, maternal pregnancy and lactation, and the growth and development of offspring for two generations were investigated (Tyl and Neeper-Bradley, 1989). F_0-generation Sprague-Dawley (CD) rats (28 per sex per group) were exposed to target concentrations of 0, 50, 150, or 450 ppm (0, 300, 900 or 2700 mg/m^3) 1,4-DCB vapor for 6 hours/day, 7 days/week, for 10 weeks before mating. The initial analytical method was found to be inadequate, resulting in an underestimation of the vapor concentrations during the first 80 days of the study. The corrected mean analytical concentrations for the three 1,4-DCB exposure groups were 66, 211, and 538 ppm (398, 1268, or 3233 mg/m^3).

The animals were mated during the next 3 weeks to produce the F_1 generation. Exposure of study females continued through mating and 19 days of gestation Exposure was discontinued from GD 20 – postnatal day (PND) 4 (date of birth was designated as PND 0), and then resumed on postnatal day 5 through weaning on postnatal day 28. During PND 5–28, mothers were removed from their litters for the daily 6-hour exposures, and then returned to their litters.

A satellite group of female rats (10 per group) were exposed concurrently to the same exposure protocol for 10 weeks. Male rats that did not successfully mate in the first 10 days were paired with the satellite females for 10 days. The study females that did not mate with males during the first 10 days of the mating period were remated with proven males from the same exposure group. For F_0 males, daily exposures continued through the study for a total of approximately 104 days (nearly 15 weeks). The total exposure duration for F_0 females was approximately 141 days (20 weeks).

Twenty-eight weanlings per sex from the F_1 generation and satellite groups of 10 F_1 females were randomly selected and exposed for 11 weeks and mated as described above to produce the F_2 generation. Liver and kidneys in all groups and selected other tissues including pituitary, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, and prostate were microscopically examined in the control and high-exposure groups.

No reproductive parameters were affected by exposure to 1,4-DCB in either generation. Clinical signs of recurrent acute toxicity were observed in 538 ppm group F_0 and F_1 adult rats throughout the exposure period. The effects included tremors, unkempt appearance, urine stains, wet fur, salivation, and periocular, perioral and perinasal encrustation. Hypoactivity and ataxia was observed to a lesser extent. Further details on these findings are presented in the Animal Acute Toxicity Section (Section 5.3).

Reductions in body weight gain were observed during most of the 10-week pre-breed exposure in 538 ppm F_0 and F_1 males, and during the first or second week of the study in the 211 ppm F_0 and F_1 males. Reduced body weight occurred occasionally in 538 ppm F_0 females during the 10-week pre-breed exposure period. During the breeding phase, maternal F_0 gestational body weight and weight gain were reduced in the 538 ppm group. Maternal F_0 body weight of the 211 ppm group was reduced approximately 5% ($p < 0.05$) compared to the control group on GD 20 However, following gestation the mean body weight of this group on lactation day 0 was similar to the control group. No developmental abnormalities were observed in examined pups. F_1 adult females exhibited reduced gestational and lactational body weights at 538 ppm during the breeding phase. No treatment-related mean body weight reduction occurred in the F_1 female 211 ppm group during gestation.

Treatment-related microscopic findings were limited to the liver and kidney. No treatment-related findings were found in the reproductive organs examined, including the vagina, uterus, ovaries, testes, epididymides, seminal vesicles, and prostate. For the kidney, hyaline droplet nephrosis was observed in all 1,4-DCB-exposed adult F_0 and F_1 male rats. For the liver, centrilobular hepatocellular hypertrophy was observed in both the high dose male and female adult rats. Further details on these findings are presented in the Chronic Inhalation Toxicity to Experimental Animals Section (Section 6.3).

Table 12. F_1 and F_2 pup litter size (mean ± SD) on lactation day 0 and 4 (PND 0 and 4) following exposure to 1,4-DCB in the Tyl and Neeper-Bradley (1989) two-generation study.

Endpoint	0 ppm (0 µg/m³)	66 ppm (398 µg/m³)	211 ppm (1268 µg/m³)	538 ppm (3233 µg/m³)
F_1 pups born/litter on Lactation day 0 (n litters)	13.9 ± 3.09 (25)	14.1 ± 1.88 (23)	12.0 ± 3.62 (27)	12.5 ± 3.81 (22)
F_1 pup total born alive/litter on Lactation day 0 (n litters)	13.0 ± 2.91 (24)	14.0 ± 1.87 (23)	11.6 ± 4.01 (27)	11.6 ± 3.86 (22)
F_1 pup litter size on Lactation day 4 – precull (n litters)	12.9 ± 2.82 (23)	13.7 ± 1.87 (23)	11.2 ± 4.13 (27)	10.5 ± 3.61* (20)
F_2 pups born/litter on Lactation day 0 (n litters)	13.5 ± 3.36 (23)	12.8 ± 3.73 (20)	13.7 ± 2.14 (24)	11.4 ± 4.25 (21)[a]
F_2 pup total born alive/litter on Lactation day 0 (n litters)	14.0 ± 1.98 (22)	12.5 ± 3.62 (20)	13.6 ± 2.10 (24)	10.7 ± 3.91** (21)
F_2 pup litter size on Lactation day 4 – precull (n litters)	13.9 ± 1.88 (22)	12.4 ± 3.69 (20)	13.5 ± 1.91 (24)	10.7 ± 3.24** (16)

[(a)] A female that was declared delivered with no pups was eliminated from the mean.
* $p < 0.05$; ** $p < 0.01$
Abbreviations: n – number; PND – post-natal day; F_1 – first generation; F_2 – second filial generation

Statistically significant fetotoxic effects in both F_1 and F_2 litters were limited to the 538 ppm exposure groups (Tables 12–14). The fetotoxic effects included increased stillborn pups and reduced total number of pups born alive per litter in the F_2 generation, reduced F_1 and F_2 pup mean litter size on PND 4 (precull), increased number of F_1 and F_2 pup deaths during PND 1–4, reduced pup body weights and weight gains per litter in both F_1 and F_2 generations, and an overall reduction in the pup survival index. F_1 and F_2 pup body weights in the 538 ppm group were significantly reduced from postnatal day 0 to 28 (Table 14).

Table 13. Key F_1 and F_2 pup viability findings at birth (PND 0) and PND 1 to 4 following exposure to 1,4-DCB in the Tyl and Neeper-Bradley (1989) two-generation study.

Exposure group	Endpoint	0 ppm (0 µg/m³)	66 ppm (398 µg/m³)	211 ppm (1268 µg/m³)	538 ppm (3233 µg/m³)
F_1 pups, PND 0[a]	Total born alive	313	323	313	256
	No. stillborn	34[b]	1**	10**	20
F_1 pups, PND 4 (precull)	No. alive	296	315	292	209
	No. died (PND 1–4)	17	8	21	47**
F_2 pups, PND 0	Total born alive	308	249	326	225
	No. stillborn	2	6	3	14**
F_2 pups, PND 4 (precull)	No. alive	305	248	323	171
	No. died (PND 1–4)	3	1	3	54**

[(a)] Date of birth was designated as PND 0

[(b)] 26 of 34 stillborn pups were from two litters

** Significantly different from control group ($p < 0.01$)

Abbreviations: PND – postnatal day; F_1 – first generation; F_2 – second filial generation

Table 14. F_1 and F_2 pup body weights per litter (in g, mean ± SD) on lactation day 0 and 28 (PND 0 and 28) following exposure to 1,4-DCB in the Tyl and Neeper-Bradley (1989) two-generation study.

Endpoint	0 ppm (0 µg/m³)	66 ppm (398 µg/m³)	211 ppm (1268 µg/m³)	538 ppm (3233 µg/m³)
F_1 pup body weight on PND 0 (n litters)	6.14 ± 0.749 (24)	5.98 ± 0.496 (23)	6.08 ± 0.704 (27)	5.37 ± 1.030** (22)
F_1 pup body weight on PND 28 (n litters)	83.87 ± 0.33 (23)	79.91 ± 7.421 (23)	82.21 ± 6.275 (25)	67.81 ± 11.345** (20)
F_2 pup body weight on PND 0 (n litters)	6.23 ± 0.470 (22)	6.32 ± 0.558 (20)	6.19 ± 0.800 (24)	5.43 ± 0.563** (19)
F_2 pup body weight on PND 28 (n litters)	83.22 ± 6.421 (22)	81.84 ± 5.535 (20)	83.79 ± 5.479 (24)	69.94 ± 7.113** (15)

** significantly different from controls groups ($p < 0.01$)

Abbreviations: PND – postnatal day; F_1 – first generation; F_2 – second filial generation

When selected control and high dose pups from the first filial generation (20 F_1 pups /sex/dose) were allowed to recover from the 1,4-DCB exposure for a 5-week period following weaning, body weights of the 538 ppm exposure group remained lower than those for the controls

No treatment-related gross observations were found in any of the F_1 or F_2 weanling rats. None of the organs in F_2 pups were microscopically examined. The study authors concluded that the NOEL for maternal toxicity was 66 ppm (for decreased maternal body weight on GD 20) and developmental toxicity in offspring was 211 ppm (for decreased body weight and increased stillborn and pup deaths during the perinatal period), indicating no increased risk to offspring in the absence of maternal effects.

Current information is inadequate to assume that developmental effects at maternally toxic doses result only from maternal toxicity. It may simply indicate both are sensitive to the same exposure level. Developmental effects at the same, or higher, exposure levels as that of maternal effects should still be considered to represent

developmental toxicity and should not be discounted as secondary to maternal toxicity (EPA, 1991).

An oral two-generation reproductive and developmental toxicity study by Bornatowicz et al. (1994) is summarized here as supportive evidence for the two-generation inhalation study [Professionally translated for OEHHA from German to English]. The oral study also conducted several neurobehavioral tests on the offspring, which has not been performed for 1,4-DCB in other animal toxicity studies. Male and female Sprague Dawley rats (24 rats/sex/dose) of the parental F_0 generation were administered 1,4-DCB via daily gavage at doses of 0, 30, 90 or 270 mg/kg-day, 7 days/week for 77 days and 21 days before mating in males and females, respectively. The males were exposed for a longer duration than females during the pre-mating phase to expose the sperm through all stages of spermatogenesis. Dosing continued in both sexes for 21 days during the mating phase, and in females during gestation (21 days). Exposure of the F_0 females continued throughout lactation until weaning of their pups (F_1 generation) on postnatal day 21. On PND 4, F_1 pups were culled to 8 pups per litter (4 males and 4 females when possible). Oral administration of 1,4-DCB began on PND 21 in F_1 rats (24 rats/sex/dose) and continued for approximately 80 days. After the pre-mating exposure, F_1 animals were mated (using the same protocol as used for the F_0 rats) to produce the F_2 generation. F_2 pups were sacrificed and examined at weaning.

There were no treatment-related effects on mating or fertility at any dose level. At necropsy, absolute and relative kidney and liver weights were increased and spleen weights were decreased in 270 mg/kg F_0 and F_1 adult males compared to the control group. The relative liver weight in 90 mg/kg F_1 adult males were also increased compared to controls. Histological examination of the reproductive system, liver, spleen, and kidneys were conducted only in rats found prematurely dead, were found in a moribund state and sacrificed, or were infertile (numbers not stated). No treatment-related lesions were found in the liver or reproductive organs of these animals. Kidney damage was observed in high dose adult rats mainly in the tubules. The authors did not explicitly state if one or both sexes exhibited the kidney affects. No significant reduction in body weights of F_0 rats were observed in the 1,4-DCB-dosed groups compared to the control group.

In clinical observations, ringtail was observed in all or many F_1 and F_2 litters in the 90 and 270 mg/kg groups (incidence not specified) and was considered treatment-related. Ringtail, or tail necrosis, is an epidermal disease in which annular constrictions occur along the length of the tail, resulting in necrosis and possible loss of the tail distal to the necrotic constriction. Low environmental humidity, dehydration, and a number of other causes have been attributed to this disease. In addition, a significant number of F_1 pups in the high dose group appeared cyanotic compared to

the control group (incidence not specified). Dry and squamous skin was also observed during the first week after birth in both F_1 and F_2 litters, with 70% and 100% of litters exhibiting this skin lesion in the 90 and 270 mg/kg groups, respectively. Dry, squamous skin was not observed in any rats in the control and low dose groups.

Some malformations in rat pups were observed at the two highest doses in both generations (one each at 90 mg/kg, and 2 each in the 270 mg/kg group) that were considered uncommon (e.g., renal ectopia). However, the authors stated that the study was not designed to make determinations of teratogenicity in offspring of treated rats.

Body weights of F_1 and F_2 offspring were significantly reduced at birth in both the 90 and 270 mg/kg groups ($p < 0.05$). The body weights of only the high dose groups remained reduced compared to controls up until the end of the lactation period (PND 21). In F_1 rats used to produce the F_2 generation, the parental body weights of the high dose males and females were significantly lower compared to controls throughout most of the study (data not shown).

The total number of pups born was not different between dosing groups in either generation. However, the total number of pups dead at birth, and total number of pups that died between PND 0 and 4, was significantly increased in the 270 mg/kg group compared to control in both F_1 and F_2 generations ($p < 0.05$). In addition, the number of dead F_2 pups in the 90 mg/kg group was also significantly increased between PND 0 and 4. F_1 and F_2 pups that died between PND 5–21 were also significantly higher in the high dose groups. The increase in dead pups resulted in a significantly reduced survival index for the high dose F_1 and F_2 generations ($p < 0.05$).

Developmental milestones including erection of ears and eye opening were measured in offspring of both generations. Neurobehavioral effects, including outer ear reflex, orientation reaction, grasping reflex, and draw-up test were measured in both F_1 and F_2 pups. The outer ear reflex tests whether ear or head flicking occurs when a brush touches the interior part of the outer ear. The orientation reaction tests whether a pup held up by the base of the tail will reach for the edge of a nearby table. The grasping reflex measures the ability to hold onto a wire with the front paws, and the draw-up test determines if the pup can reach the wire with at least one hind leg while holding onto the wire with front paws. For developmental milestones, the day in which all pups per litter showed erection of ears was significantly delayed in 270 mg/kg F_2 pups compared to the control group ($p < 0.05$). The first day of eye opening per litter was significantly delayed in high dose F_1 and F_2 pups, as was the day in which all F_2 pups per litter showed this effect. For neurobehavioral effects, a significantly lower percentage ($p < 0.05$) of 270 mg/kg F_1 and F_2 pups per litter were

able to accomplish the draw-up reflex (77% versus 95% for F_1 control versus F_1 270 mg/kg pups, respectively; 73% versus 94% for F_2 control versus F_2 270 mg/kg pups, respectively). No treatment-related effects were seen for the other three neurobehavioral tests.

Table 15 summarizes animal studies relevant for reproductive and developmental endpoints. In general, a developmental study in rabbits observed one anomaly (increased incidence of retroesophageal right subclavian artery) in offspring at the highest exposure level (800 ppm), and a non-dose-related increase in resorbed implantations. A two-generation inhalation reproduction and developmental study in rats observed primarily reduced body weight, litter size and decreased viability in F_1 and F_2 offspring in the high exposure groups (538 ppm). Body weights in F_0 and F_1 adults were reduced in the high exposure groups. A two-generation oral (gavage) study in rats also observed reduced body weight and viability in F_1 and F_2 offspring, in addition to delayed developmental milestones in offspring and reduced neurobehavioral performance.

1633 **Table 15. Summary of developmental and reproductive effects of 1,4-DCB exposure in experimental animals.**

Reference	Animal model and exposure	Results	Point of Departure
Hodge et al. (1977), as reported in US EPA (1989)	SPF Alderly Park female rats exposed via inhalation to 0, 75, 200 and 500 ppm 6 hours/day during GD 6–15	No exposure related effects on maternal toxicity, embryotoxicity, fetotoxicity, or teratogenicity	NOAEL: 500 ppm LOAEL: NA
Hayes et al. (1985) Hayes et al. (1982)	Female New Zealand white rabbits exposed via inhalation to 0, 100, 300 or 800 ppm for 6 hours/day during GD 6–18.	↓ maternal BW on GD 6–8 at 800 ppm ↑ incidence of retroesophageal right subclavian artery in fetuses at 300 ppm ↑ percentage of resorbed implantations and litters with resorptions at 300 ppm, but not at 800 ppm	NOAEL= 300 ppm LOAEL = 800 ppm for increased incidence of retroesophageal right subclavian artery
Tyl and Neeper-Bradley (1989)	Two-generation study in male and female Sprague-Dawley rats exposed via inhalation to 0, 66, 211 or 538 ppm (28 rats/sex/group) for 6 hours/day, 7 days/week for 15 weeks in F_0 males and 20 weeks in F_0 females covering pre-mating, mating, and gestation/lactation (females only) phases. Similar protocol used for F_1 rats although total exposures were 21–22 weeks	Consistent ↓ BW in 538 ppm F_0 and F_1 males ↓ F_0 maternal BW at 538 ppm during gestation, and at 211 ppm on GD 20 ↓ F_1 maternal BW at 538 ppm during gestation and lactation ↓ F_1 and F_2 pup litter size at 538 ppm ↓ F_1 and F_2 pup BW and weight gain at 538 ppm ↑ stillborn pups (F_2) and pup deaths on PND 1–4 (F_1 and F_2) at 538 ppm	NOAEL: 211 ppm LOAEL:538 ppm for developmental toxicity
Bornatowicz et al. (1994)	Two-generation study in male and female Sprague-Dawley rats exposed via oral gavage to 0, 30, 90, or 270 mg/kg-day for at least 14 weeks in F_0	↓ F_1 and F_2 pup BW only at birth at 90 mg/kg, and during entire lactation period at 270 mg/kg	NOAEL: 30 mg/kg-day

Reference	Animal model and exposure	Results	Point of Departure
	males and 12 weeks in F_0 females covering pre-mating, mating, and gestation-lactation (females only) phases. Similar protocol used for F_1 rats although total exposures were at least 14.5 weeks in males and 20 weeks in females	↑ F_1 and F_2 stillborn pups and pup deaths during PND 1–4 and PND 5–21 at 270 mg/kg ↑ F_2 pup deaths during PND 1–4 at 90 mg/kg ↑ F_1 and F_2 pups with ringtail and dry, squamous skin at 90 and 270 mg/kg ↑ F_1 pups that appeared cyanotic at birth at 270 mg/kg Delayed eye opening in F_1 and F_2 pups and delayed ear erection in F_2 pups at 270 mg/kg ↓ F_1 and F_2 pup neurobehavioral performance in draw-up test at 270 mg/kg	LOAEL: 90 mg/kg-day for developmental toxicity only

Abbreviations: ↓ – decreased significantly ($p < 0.05$) relative to control; ↑ – increased significantly ($p < 0.05$) relative to control; BW – body weight; F_1 – first offspring generation; F_2 – second filial generation; F_0 – parental generation; GD – gestation day; LOAEL – Lowest Observed Adverse Effect Level; NA – not applicable; NOAEL – No Observed Adverse Effect Level; PND – postnatal day; ppm – parts per million.

8. Derivation of Reference Exposure Levels

8.1 1,4-Dichlorobenzene Acute Reference Exposure Level

Study	Tyl and Neeper-Bradley (1989)
Study population	Pregnant Sprague-Dawley rats
Exposure method	Whole-body inhalation
Exposure continuity	Exposure to 0, 398, 1,268 or 3,233 mg/m^3 (0, 66, 211, or 538 ppm)
Exposure duration	6 hours/day, 7 days/week in F_0 and F_1 females covering pre-mating, mating and gestation-lactation phases (with no exposure on PND 1–4)
Critical effects	Decreased viability in F_2 generation rat pups
LOAEL	3,233 mg/m^3 (538 ppm)
NOAEL	1,268 mg/m^3 (211 ppm)
Benchmark concentration	1,731 mg/m^3 (288 ppm)
Time-adjusted exposure	1,731 mg/m^3 (288 ppm) (No time adjustment for developmental effects)
Human Equivalent Concentration (HEC)	1,731 mg/m^3 (288 ppm), given a Regional Gas Dose Ratio (RGDR) = 1[a]
LOAEL Uncertainty Factor (UF_L)	1
Interspecies Toxicokinetic Uncertainty Factor (UF_{A-k})	2
Interspecies Toxicodynamic Uncertainty Factor (UF_{A-d})	√10 (default)
Intraspecies Toxicokinetic Uncertainty Factor (UF_{H-k})	10 (systemic toxicant)
Intraspecies Toxicodynamic Uncertainty Factor (UF_{H-d})	√10 (default)
Cumulative uncertainty factor	200
Acute Reference Exposure Level	**8.7 mg/m^3 (8,700 µg/m^3; 1.5 ppm; 1,500 ppb)**

[(a)] The default value for the RGDR is 1 for a systemic effect, including maternal exposure resulting in developmental effects in offspring (OEHHA, 2008).

Abbreviations: F_0 – parental generation; F_1 – first offspring generation; F_2 – second filial generation; LOAEL – Lowest Observed Adverse Effect Level; mg/m^3 – milligrams per cubic meter; µg/m^3 – micrograms per cubic meter; NOAEL – No Observed Adverse Effect Level PND – postnatal day; ppb – parts per billion; ppm – parts per million.

The acute Reference Exposure Level (REL) is a level at which infrequent one-hour exposures to 1,4-DCB are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (OEHHA, 2008).

Only a limited number of 1,4-DCB acute exposure studies in humans or animals are available. In an occupational study, daily exposures to 15–85 ppm (average: 45 ppm) did not cause complaints, whereas daily exposures to 50–170 ppm (average: 105 ppm) resulted in sensory irritation (Hollingsworth et al., 1956). However, this study was inadequate for derivation of an acute REL. In animals, observation of rats exposed to an estimated 571 ppm for 6 hours on the first day of a two-generation study resulted in sensory irritation, including periocular, perinasal and perioral encrustation (Tyl and Neeper-Bradley, 1989). Subjective observations of possible neurotoxicity in the form of tremors was also noted on the first day of exposure. Similar signs of toxicity were observed by Hollingsworth et al. (1956) in rats, guinea pigs and rabbits exposed 8 hours per day to 798 ppm 1,4-DCB over multiple days, although it was unclear if the toxic effects were observed on the first day of exposure.

A stronger basis for acute REL derivation is found with 1,4-DCB animal exposure studies during development. Even though daily exposures occur over multiple days during gestation, a single exposure for as short as one hour at any of several developmental stages may be sufficient to produce an adverse effect (EPA, 1991; OEHHA, 2008). Developmental effects that were considered for acute REL derivation included increased incidence of retroesophageal right subclavian artery in fetal rabbits (Hayes et al., 1985), and decreased rat pup viability and body weights in a two-generation exposure study (Tyl and Neeper-Bradley, 1989).

The significantly increased incidence of retroesophageal right subclavian artery in 800 ppm rabbit fetuses was not considered by Hayes et al. (1985) to be a result of 1,4-DCB exposure during development, primarily due to the presence of this variation in 2% of their laboratory historical controls. No other information regarding their historical control data was provided. OEHHA considers this blood vessel anomaly in fetal rabbits to be a result of maternal exposure to 1,4-DCB. While OEHHA acknowledges the possibility of a type I error (i.e., a false positive) for the anomaly, the significantly increased incidence on both a per-fetus and per-litter basis in the 800 ppm group compared to the concurrent control group is strongly supportive of a chemically-related effect. In particular, the distribution of six fetuses with the anomaly over five litters is stronger evidence for a true effect, as compared to six affected fetuses in one litter.

In the absence of certainty, OEHHA takes the health protective approach based on reduced fetal body weight in animal fetuses. The logarithm of infant mortality in humans increases linearly as birth weight decreases from 3500 to 1000 grams with

no evidence for a threshold (Hogue et al., 1987; Rees and Hattis, 1994). Thus, any reduction in fetal weight is a cause for concern since it increases risk of mortality. OEHHA considers the decreased body weight in 1,4-DCB-exposed rat fetuses to be adverse and treatment-related.

Benchmark dose (BMD) analysis (version 3.3.2) was performed on all adverse developmental endpoints in the animal fetuses and offspring (EPA, 2023). Only the highest exposure concentration resulted in a statistically significant increase of an adverse effect, with the next lowest exposure showing results similar to that of the control group. Studies with only a single dose showing a response different from controls may not support BMD analysis, although if the one elevated response is near the BMR, adequate BMD computation may result (Kavlock et al., 1996; EPA, 2012). For endpoints not amenable to BMD analysis, a standard NOAEL/LOAEL approach would be used. For exposure to airborne toxicants such as 1,4-DCB, benchmark modeling will be expressed as benchmark concentration (BMC).

For developmental alterations such as retroesophageal right subclavian artery, a BMR of 5% is generally used in dichotomous BMC modeling (OEHHA, 2008). The increased incidence of this soft tissue alteration was 5% in the rabbit fetuses (6/119 fetuses) of the 800 ppm (4,808 mg/m^3) group (Hayes et al., 1985). Although there was a statistically significant increase in this alteration ($p \leq 0.05$), the incidence was too low for adequate BMC modeling with a BMR of 5%. An additional consideration for not applying the BMC approach is that only a single dose level (800 ppm) shows a response different from controls. Thus, the NOAEL/LOAEL approach was applied to this data set, resulting in a LOAEL of 300 ppm and a LOAEL of 800 ppm.

Table 16 summarizes the BMC results for the adverse developmental endpoints in the two-generation inhalation study (Tyl and Neeper-Bradley, 1989). For decreased F_1 and F_2 pup body weight, continuous BMC models with a BMR of 1 standard deviation of the control mean (1SD) are employed by OEHHA for estimating the Point of Departure (POD). The lowest $BMCL_{1SD}$ of 345 ppm (2,073 mg/m^3) was attained for decreased birth weight in the F_2 rat pups. The $BMCL_{1SD}$ represents the 95% lower confidence limit of the BMC.

The nested logistic model provided by US EPA (2023) was used to determine the POD for dichotomous endpoints, including stillborn pups at birth and total dead pups out to PND 4. This is the period (birth to PND 4) in which the mothers were not exposed to 1,4-DCB. Access to individual animal data for these endpoints allows the use of the nested logistic model. The benchmark response (BMR) of 5% extra risk was used to derive the BMC and $BMCL_{05}$ for dichotomous data. The BMC is the dose at the 5% response rate, and the $BMCL_{05}$ represents the 95% lower confidence limit of the dose producing a 5% response rate.

Litter size was the litter-specific covariate (lsc) for this analysis, which is a commonly used lsc provided no treatment-related resorptions and prenatal deaths occurred (US EPA, 2012). The number of implantation sites per litter is another lsc that is used in nested modeling, if available, but was not assessed in the two-generation study. The number of pups born per litter in both F_1 and F_2 generations was not affected by maternal 1,4-DCB exposure, but it is not known if the implant numbers differed among dose groups.

BMD nested analysis on the number of stillborn F_1 pups at birth and dead F_1 pups during PND 1–4 was not determined, even though there appeared to be an increase in pup deaths at the highest concentration. A high number of stillborn pups were born in the F_1 control group, primarily from two litters (26 of 34 stillborn control pups, See Table 13). The authors did not explain the potential cause of these deaths. The nested dichotomous results for the rat pup viability endpoints that had acceptable model fits to the data are summarized in Table 16. The model with lowest POD (and lowest Akaike Information Criterion (AIC)) is the combined stillborn and dead F_2 pups during PND 0–4 in which the lsc is not included. In this model the intra-litter correlation (ilc) is an important factor, indicating more similarity in pups within the same litter than pups in different litters. This decrease in pup viability in F_2 pups (PND 0–4) provided the most health protective POD for the developmental endpoints shown in Table 16.

BMC modeling of the continuous data for other endpoints with treatment-related effects (F_1 and F_2 pup litter size at PND 4, and total F_2 pups born alive per litter) did not improve the fit to the data observed with modeling of the dichotomous data and had some additional limitations ($p < 0.1$ for model fit, BMC higher than highest exposure group). Therefore, these BMC results are not discussed further.

Table 16. Summary of BMC results for decreased body weight and viability in F_1 and F_2 rat pups from the two-generation 1,4-DCB inhalation study (Tyl and Neeper-Bradley, 1989).

Endpoint	Model	BMC[(a)] (ppm)	BMCL[(b)] (ppm)	*p*-value	AIC
F_1 pup decreased body weight (PND 0)	Polynomial deg3 (NCV)	547*	431	0.12	220.91
F_2 pup decreased body weight (PND 0)	Polynomial deg2 (CV)	452	345	0.82	161.81
F_2 Stillborn pups (PND 0)	Nested lsc+, ilc-	564*	506	0.12	230.99
	Nested lsc-, ilc+	546*	476	0.21	231.37
F_2 Stillborn + dead pups (PND 0–4)	Nested lsc+, ilc+	467	293	0.11	374.40
	Nested lsc-, ilc+	464	288	0.11	371.13

[(a)] Benchmark concentration at 1 standard deviation (SD) from the control group mean for decreased pup body weight, and benchmark concentration at the 5% response rate for stillborn and stillborn + dead pup results.

[(b)] The 95% lower confidence limit of the concentration that is 1 SD from the control group mean (decreased pup body weight), or that produces a 5% response rate (stillborn and stillborn + dead pups).

* BMC higher than highest exposure group (538 ppm)

Abbreviations: AIC: Akaike information criterion; CV: constant variance; ilc: intra-litter correlation; lsc: litter specific covariate; NCV: non-constant variance; PND: postnatal day

Supporting data for the Acute REL includes the two-generation gavage study in rats by Bornatowicz et al. (1994), in which 1,4-DCB exposure also resulted in decreased body weight and viability in both F_1 and F_2 generation pups. In human population surveys, an increase in the urinary metabolite 2,5-DCP in pregnant women was found to be associated with lower birth weight in male infants (Wolff et al., 2008; Philippat et al., 2012). Increased urinary levels of 2,5-DCP in pregnant women has also been associated with increased odds of respiratory and allergic outcomes in their young boys (Buckley et al., 2018). Other surveys have observed associations of earlier onset of puberty in girls with higher 2,5-DCP levels in their urine, suggesting that 1,4-DCB may alter hormonal activity in children (Buttke et al., 2012; Wolff et al., 2015; Wolff et al., 2017).

No temporal adjustment was used to modify the PODs since the critical period of exposure for a developmental effect may be very short relative to the study duration (OEHHA, 2008). For a systemic effect, including maternal exposure resulting in developmental effects in offspring, the default value for the Regional Gas Dose Ratio (RGDR) is 1. This value assumes the blood:air coefficient is the same across species. Supporting pharmacokinetic evidence by Yoshida et al. (2002b) estimated that daily inhalation absorption rates of DCB were similar in rats and humans.

Similarities in metabolism and excretion have been observed in rat and human pharmacokinetic studies (Fisher et al., 1995; Yoshida et al., 2002a; Yoshida et al., 2002b). As a result, an Interspecies Pharmacokinetic Uncertainty Factor ($UF_{A\text{-}k}$) of 2 was applied to reflect remaining uncertainties due to metabolism and excretion. A default $UF_{A\text{-}d}$ of $\sqrt{10}$ was applied to account for pharmacodynamics or response differences between species. The default intraspecies toxicokinetic $UF_{H\text{-}k}$ of 10 is applied for gases that act systemically and to address variability within the human population (OEHHA, 2008). Several population studies observed hormonal, respiratory, and neurotoxic effects in newborns and children that were associated with increased exposure to 1,4-DCB (primarily as the 2,5-DCP metabolite in urine). However, since the critical study was based on a sensitive endpoint (development) the default intraspecies toxicodynamic $UF_{H\text{-}d}$ of $\sqrt{10}$ was appropriate for REL derivation. The cumulative UF = 200 applied to the HEC-adjusted POD of 1,731 mg/m^3 (288 ppm) results in an acute REL = 8.7 mg/m^3 (1.5 ppm), which rounds to 9 mg/m^3 (1.5 ppm) in the final assessment.

The high dose exposure group was the only elevated response for the developmental endpoints, which is not ideal for BMC analysis. However, the response level in the high dose group was near the BMR for the pup viability and pup body weight results, indicating that BMC analysis may have an advantage over the conventional NOAEL/LOAEL approach. Derivation of alternate REL values using the POD of 345 ppm for decreased rat F_2 pup body weight, and the POD of 300 ppm for the blood vessel anomaly in fetal rabbits results in an alternate REL values of 1.7 ppm and 1.5 ppm, respectively. These REL values are similar to the Acute REL based on decreased rat pup viability (1.5 ppm). Therefore, all three endpoints should be considered critical developmental endpoints for the Acute REL.

The acute REL will be protective for sensory irritation and possible neurotoxicity also observed in the high exposure rats. Overlooking the methodology limitations in the Hollingsworth et al. (1956) occupational study, the Acute REL is over 10 times lower than the presumed NOAEL of 45 ppm for sensory irritation in the workers.

8.2 1,4-Dichlorobenzene Chronic Reference Exposure Level

Study	Aiso et al. (2005b)
Study population	Groups of 50 male and female F344/DuCrj rats
Exposure method	Inhalation exposure to 0, 120, 450, and 1,800 mg/m^3 (0, 20, 75, and 300 ppm)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Critical effects	Degenerative changes in the nasal olfactory epithelium
LOAEL	450 mg/m^3 (75 ppm)
NOAEL	120 mg/m^3 (20 ppm)
Benchmark Concentration	27.95 mg/m^3 (4.65 ppm)
Time-adjusted exposure	4.99 mg/m^3 (0.83 ppm) - 6 hours/24 hours × 5 days/7 days)
Human equivalent concentration	0.998 mg/m^3 (0.166 ppm) (0.83 ppm × 0.2; RGDR for extrathoracic respiratory effects)
LOAEL Uncertainty Factor (UF_L)	1
Subchronic Uncertainty Factor (UF_S)	1
Interspecies Toxicokinetic Uncertainty Factor (UF_{A-k})	2 (for residual toxicokinetic differences)
Interspecies Toxicodynamic Uncertainty Factor (UF_{A-d})	√10 (no interspecies toxicodynamic data)
Intraspecies Toxicokinetic Uncertainty Factor (UF_{H-k})	10 (to allow for intra human diversity, including infants and children)
Intraspecies Toxicodynamic Uncertainty Factor (UF_{H-d})	√10 (default)
Cumulative uncertainty factor	200
Chronic Reference Exposure Level	**5.0 $\mu g/m^3$ (0.8 ppb)**

Abbreviations: LOAEL – Lowest Observed Adverse Effect Level; mg/m^3 – milligrams per cubic meter; $\mu g/m^3$ – micrograms per cubic meter; NOAEL – No Observed Adverse Effect Level; ppb – parts per billion; ppm – parts per million; RGDR – Regional Gas Dose Ratio.

The chronic REL is a concentration at which adverse noncancer health effects would not be expected in the general population exposed continuously over a lifetime (see Section 7 in the Technical Support Document (OEHHA, 2008)). The derivation of the

chronic REL for 1,4-DCB is based on the 2-year chronic toxicity/carcinogenicity study in F344/DuCrj rats and Crj:BDF1 mice (Aiso et al., 2005b). Tables 8 and 9 summarize the noncancer pathology findings from the study. The primary organ systems affected in the 1,4-DCB-exposed rodents included the upper respiratory system, liver, kidney, and the male reproductive system.

In the upper respiratory tract, there was a dose-related increased incidence of eosinophilic globules (moderate and marked severity levels combined) in nasal olfactory epithelium of female rats that was significantly greater in the 75 and 300 ppm 1,4-DCB groups compared to the control group. The presence of eosinophilic globules have been described as a degenerative change seen in sustentacular cells of the olfactory epithelium, respiratory epithelial cells, and epithelium of the nasal seromucous glands (Renne et al., 2003; Harkema et al., 2006; Renne et al., 2009). The globules contain proteinaceous material in membrane-bound vacuoles and cause the affected cells to become markedly dilated. They increase in size and number in nasal epithelium of rats following exposure to toxic agents or as a consequence of ageing. Renne et al. (2009) stated that eosinophilic globules are a prominent feature of all types of epithelial hyperplasia, but are also seen in non-hyperplastic cells. The incidence of eosinophilic globules in olfactory epithelium in normal ageing rodents was lower in mice when compared to rats (Nagano et al., 1997).

A significant increase ($p < 0.05$) in mineralization of the testis in male mice was reported in the 75 and 300 ppm 1,4-DCB exposure groups in the summary report of the original Japanese study (JBRC, 1995). However, the implication of this lesion was not discussed in the JBRC report, and Aiso et al. (2005b) did not present the testicular mineralization incidence data in the peer-reviewed published study. In a written communication to authors of the Agency for Toxic Substances and Disease Registry (ATSDR, 2006) report on dichlorobenzenes, Dr. Aiso did not consider testicular mineralization to be a toxicologically significant effect because, (1) no signs of testicular toxicity were observed in male mice in the 13-week 1,4-DCB exposure study (Aiso et al., 2005a), and (2) the lesion was confined to the testicular capsules and blood vessels and not observed in the testicular parenchyma, indicating that it is a finding commonly observed in aged mice independent of exposure to 1,4-DCB. ATSDR (2006) agreed with this finding and did not model testicular mineralization for their dose-response assessment.

Other pathologists have also described testicular mineralization as an age-related disease, which may involve the capsule, blood vessels, or seminiferous tubules (Creasy et al., 2012; NTP, 2014). It is often an outcome of sperm stasis within the seminiferous tubules. The lesion is characterized as an accumulation of basophilic fine to coarsely granular to amorphous laminated material, with or without distortion

of the tissue architecture. The incidence of testis mineralization in aging male mice were observed to be 0.5% in the $B6C3F_1$ strain and 1.8% in the CD-1 strain (Gordon et al., 1996). Spontaneous appearance of testis mineralization was considerably greater in the aged Crj:BDF1 male mouse strain (27/49, 55%) examined in the two-year 1,4-DCB inhalation study (JBRC, 1995). The incidence range for this lesion from historical control data in Crj:BDF1 male mice was not provided.

A similar testicular lesion was observed in a two-year National Toxicology Program (NTP) rodent study of formamide (NTP, 2008). A dose-related increase in testis artery and testis tunic mineralization occurred in male mice that was statistically significant in high dose mice compared to the control group. These were the only testicular lesions observed and were considered to be treatment-related. Abnormal residual bodies were observed in testis of exposed male mice in the 3-month study that preceded the 2-year study.

Significantly increased testis mineralization in JBRC (1995) was below the 1% significance level in the 75 ppm ($p = 0.002$) and 300 ppm ($p = 0.004$) 1,4-DCB groups compared to the control group (by Fisher exact test conducted by OEHHA). In the absence of historical data to suggest otherwise, the high incidence rate in 1,4-DCB-treated male mice reduces the chance of a Type 1 error (i.e., a false positive) (Haseman, 1983; 1990). However, concurrent control data typically takes precedence over historical control data (US EPA, 1991).

Significantly increased incidences ($p < 0.01$) of male rat kidney papilla mineralization and pelvic urothelial hyperplasia were observed at the highest exposure in the two-year study (Aiso et al., 2005b). It was not indicated if this finding may be related to the excessive accumulation of α-2μ-globulin in the proximal tubules of 1,4-DCB-exposed male rats observed in their 13-week study (Aiso et al., 2005a). α-2μ-Globulin nephropathy occurs exclusively in male rats and is caused by a variety of chemicals, including 1,4-DCB (IARC, 1999). Aiso et al. (2005b) stated that this protein declines in the kidneys of 1,4-DCB-exposed male rats as they age, which is why it was absent in their two-year study.

IARC (1999) indicates that both papilla mineralization and cellular proliferation in the kidneys of male rats are the result of chronic exposure to chemicals that induce α-2μ-globulin nephropathy. In addition, no evidence of 1,4-DCB-induced nephrotoxicity was found in mice or female rats of the two-year inhalation study (Aiso et al., 2005b). Therefore, the kidney lesions caused by 1,4-DCB exposure in male rats are probably not relevant to humans. Regardless of the α-2μ-globulin nephropathy issue and whether it is relevant to humans, the BMC results in Table 17 indicate that the POD for kidney papilla mineralization and pelvic urothelial hyperplasia are well above (6-fold or greater) the POD for female rat nasal epithelial injury and male mice testicular

mineralization. Therefore, kidney toxicity was not listed as a critical endpoint of chronic inhalation of 1,4-DCB.

The treatment-related increased incidence of centrilobular hypertrophy of the liver was not considered for REL derivation. Since no histopathological evidence of hepatocellular injury was observed in any of the 1,4-DCB-exposed rats and mice in the two-year study by Aiso et al. (2005b), liver toxicity was not considered a critical effect for chronic inhalation of 1,4-DCB.

BMC analysis (EPA, 2023) of the pathology incidence data was carried out to obtain the BMC and $BMCL_{05}$ (the 95% lower confidence interval on a 5% change in the quantal endpoint) for each toxic endpoint (Table 17). Among the set of dichotomous models available, the one chosen for each modeling run of a dataset is based on recommendations by US EPA (2012), i.e., lowest AIC value, p-value for goodness-of-fit >0.1, consideration for local fit in the region on the BMCL, and best visual fit of the modeled curve to the data. For the nasal olfactory epithelial lesion in female rats, acceptable BMC model fits to the data was only achieved by combining the incidence of moderate and marked severity grades of eosinophilic globules; BMC modeling of moderate or marked grades separately did not result in acceptable BMC model fits.

Table 17. Summary of BMC and $BMCL_{05}$ for key pathology endpoints from the two-year 1,4-DCB inhalation study in rodents (Aiso et al., 2005b).

Sex and species	Endpoint	Recommended BMC model	BMC	$BMCL_{05}$	*p*-value	AIC
Female rats	Combined moderate and marked eosinophilic globules in olfactory epithelium	Multistage Degree 1	6.89	4.65	0.91	217.14
	Respiratory eosinophilic globules	Logistic	28.79	23.19	0.80	221.705
	Respiratory metaplasia of nasal gland*	Multistage Degree 3	111.95	44.35	0.95	154.728
Male rats	Kidney papilla mineralization*	Weibull	246.91	91.80	0.37	63.154
	Kidney pelvic urothelial hyperplasia	Probit	36.10	29.36	0.95	211.238
Female mice	Olfactory respiratory metaplasia*	Multistage Degree 3	151.40	74.77	0.40	166.984
Male mice	Mineralization of testis	Log-logistic	5.67	2.29	0.62	221.922

* Only the highest exposure group was significantly elevated, with all other exposure groups similar to that of the control group. Endpoints with only a single exposure concentration showing a response different from controls may not support BMD analysis.

Abbreviations: AIC: Akaike information criterion; BMC: benchmark concentration that produces a 5% response rate; $BMCL_{05}$: 95% lower confidence limit of the concentration that produces a 5% response rate.

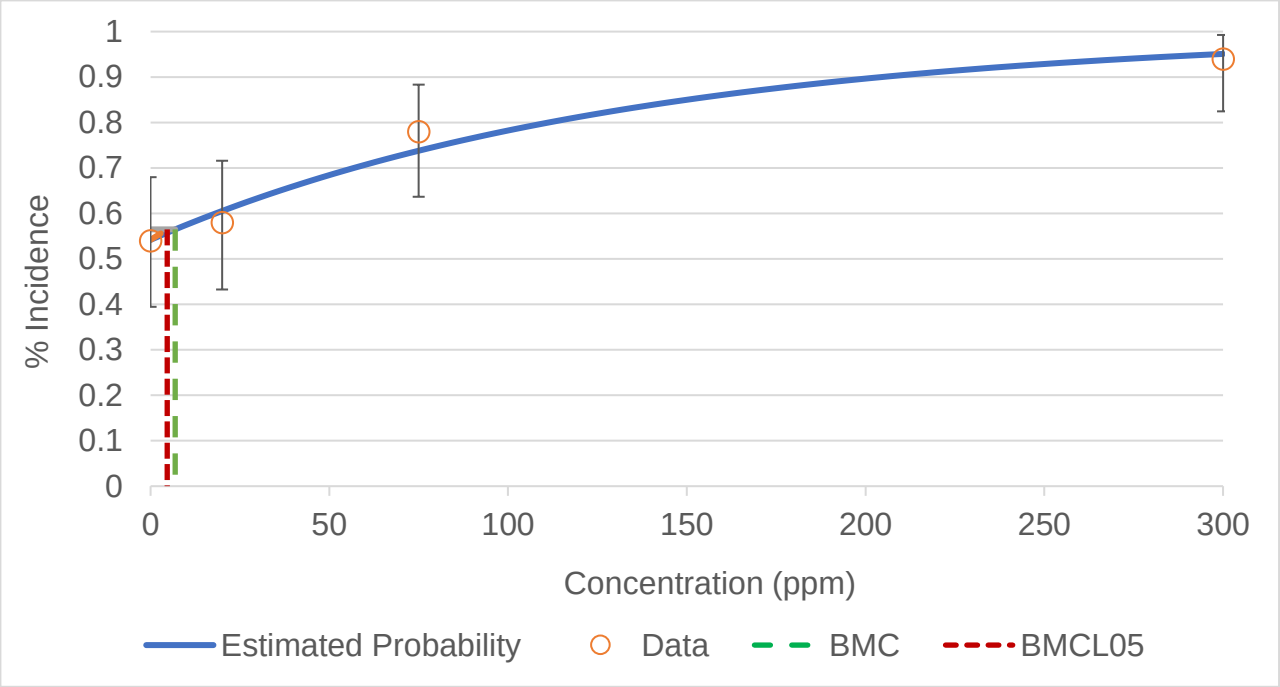

Figure 2. Multistage Degree 1 model fit to Aiso et al. (2005b) incidence data for nasal olfactory epithelial lesions of moderate or marked severity (combined) in female rats. In the graph, ppm is shown on the x-axis, and fraction affected is shown in grams on the y-axis. The open orange circles represent the original data points. The solid blue line and horizontal, dashed, green line represent the estimated probability and the concentration resulting in a 5% response (BMC; 6.89 ppm) respectively. The vertical red dashed line represents the BMC05 (4.65 ppm).

The lowest $BMCL_{05}$ in Table 17 was 2.29 ppm for mineralization of testis in male mice. However, the RGDR for nasal olfactory epithelium changes in female rats was calculated to be 0.20, whereas the RGDR for mineralization of testis in male mice was 1.0. Incorporation of the HEC value with the corresponding toxic endpoint (and including uncertainty factors) resulted in the nasal olfactory epithelium changes in female rats as the most sensitive endpoint for chronic inhalation of 1,4-DCB.

The RGDR was calculated using US EPA Human Equivalent Concentration (HEC) methodology for dosimetric interspecies extrapolation (OEHHA, 2008). For gases with respiratory system effects, the RGDR is determined as the relative minute volume to relative surface area for the lung region of concern (i.e., the upper respiratory, or extrathoracic, region).

$$RGDR = (MV_a/MV_h) / (SA_a/SA_h)$$

Where:

SA_h = human surface area for lung region (Table F.1.1, OEHHA, 2008)

SA_a = animal (rat) surface area for lung region (Table F.1.1, OEHHA, 2008)

MV_a = animal (rat) minute volume

MV_h = human minute volume

The average female rat body weight (0.3 kg) from Aiso et al. (2005b) is used to determine the minute volume with an algorithm in which allometric relationships are known for specific species (OEHHA, 2008). Minute volume of adult humans was based on the standard 20 m^3/day inhalation rate. Based on these inputs the RGDR were 0.20. For inhaled gases leading to a systemic effect, including testis mineralization, the RGDR default value is equal to one (OEHHA, 2008).

An interspecies uncertainty factor of 2 for toxicokinetic (UF_{A-k}) variability was used for residual toxicokinetic differences in studies of non-primate species using the HEC approach, while a default interspecies UF_{A-d} of $\sqrt{10}$ for toxicodynamic differences was used to reflect the lack of interspecies toxicodynamic data (OEHHA, 2008).

Although causal relationships between 1,4-DCB exposure and associations with reported health conditions in population surveys are inherently difficult to establish, numerous studies have suggested exposure to 1,4-DCB is associated with various effects on infants and children (Phillipat et al., 2012; Buckley et al., 2018; Twum and Wei, 2011; Buttke et al., 2012; Wolff et al., 2015; Wei and Zhu, 2016c; Wolff et al., 2017). In this assessment, OEHHA used an intraspecies toxicokinetic uncertainty factor (UF_{H-k}) of 10, to account for the population variability in kinetics factors including differences among infants, children, and adults. A total intraspecies UF of 30 is used to account for potential increased susceptibility of children.

The resulting cumulative UF was 200, when divided into the adjusted POD of 0.998 mg/m^3 (0.166 ppm), resulted in a chronic REL of 4.99 µg/m^3 (0.8 ppb) for 1,4-DCB – rounded to 5.0 µg/m^3 (0.8 ppb) in the final assessment. This chronic REL supersedes the previous chronic REL of 800 µg/m^3 (100 ppb) derived in 2000 and based on the two-generation inhalation reproductive study by Tyl and Neeper-Bradley (1989).

For comparison, the $BMCL_{05}$ for male mouse testis mineralization was 2.29 ppm (13.76 mg/m^3). Deriving the POD using the same time adjustment and UFs as that used for nasal olfactory epithelium degeneration, but applying the systemic default RGDR of one, a 1,4-DCB chronic REL of 2.0 ppb (12.3 µg/m^3) is obtained. This value is comparable to the chronic REL based on nasal olfactory epithelium degeneration. Therefore, male reproductive system toxicity is also considered a critical endpoint.

8.3 1,4-Dichlorobenzene 8-Hour Reference Exposure Level

Study	Aiso et al. (2005b)
Study population	Groups of 50 male and female F344/DuCrj rats
Exposure method	Inhalation exposure to 0, 120, 450, and 1,800 mg/m^3 (0, 20,75, and 300 ppm)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Critical effects	Degenerative changes in the nasal olfactory epithelium
LOAEL	450.75 mg/m^3 (75 ppm)
NOAEL	120 mg/m^3 (20 ppm)
Benchmark Concentration (BMC)	27.95 mg/m^3 (4.65 ppm)
Time-adjusted BMC	9.98 mg/m^3 (1.66 ppm) - 6 hours/24hours × 5 days/7 days × 20 m^3/10m^3
Human equivalent concentration	1.996 mg/m^3 (0.332 ppm) (1.66 ppm × 0.2; RGDR for extrathoracic respiratory effects)
LOAEL Uncertainty Factor (UF_L)	1
Subchronic Uncertainty Factor (UF_S)	1
Interspecies Toxicokinetic Uncertainty Factor (UF_{A-k})	2 (default: for residual toxicokinetic differences in studies of non-primate species using the HEC approach)
Interspecies Toxicodynamic Uncertainty Factor (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies Toxicokinetic Uncertainty Factor (UF_{H-k})	10 (to allow for intra human diversity, including infants and children)
Intraspecies Toxicodynamic Uncertainty Factor (UF_{H-d})	√10
Cumulative uncertainty factor	200
8-Hour Reference Exposure Level	**10 µg/m^3 (1.7 ppb)**

Abbreviations: LOAEL – Lowest Observed Adverse Effect Level; mg/m^3 – milligrams per cubic meter; µg/m^3 – micrograms per cubic meter; NOAEL – No Observed Adverse Effect Level; ppb – parts per billion; ppm – parts per million; RGDR – Regional Gas Dose Ratio.

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures

(see Section 6 in the Technical Support Document). Typically, the 8-hour REL addresses the intermittent exposures of offsite workers exposed to facility emissions during their work hours.

Due to the chronic nature of exposure, the only difference between the chronic REL and 8-hour REL derivation is in the time-adjusted BMC. The time-weighted average concentration for the 8-hour REL assumes that half of the 20 m^3 of air breathed every day (i.e.,10 m^3) is breathed while active at work. This time adjustment yields an extrapolated 8-hour 1,4-DCB concentration of 9.98 mg/m^3 (1.66 ppm) as the BMC. The same UFs and RGDR rationale as used in the derivation of the chronic REL are applied resulting in an 8-hour 1,4-DCB REL of 3.33 $\mu g/m^3$ (0.55 ppb), rounded to 3.3 $\mu g/m^3$ (0.6 ppb) in the final assessment.

8.4 1,4-Dichlorobenzene Health Values Derived by Other US Agencies

US EPA (1994) derived a Reference Concentration (RfC) for 1,4-DCB of 0.8 mg/m^3 based on increased liver weights in F_0 male rats from the two-generation reproductive study by Tyl and Neeper-Bradley (1989). The assessment applied an RGDR of one and a total UF of 100 to the NOAEL of 75 mg/m^3 to obtain the RfC of 0.75 mg/m^3 (rounded up to 0.8 mg/m^3). An intraspecies UF = 10 was used to account for variability in the human population, including sensitive subpopulations, an interspecies factor of 3 was used for differences not accounted for by the HEC, and a subchronic-to-chronic UF of 3 was used since the NOAEL was based on a sub-chronic study. OEHHA adopted this value as a chronic REL for the Air Toxics Hot Spots Program in 2000, prior to being superseded by the chronic REL in the present document.

ATSDR (2006) developed Minimal Risk Levels (MRLs) for 1,4-DCB. MRLs are intended only to serve as a screening tool to help public health professionals to identify contaminants and potential health effects that may be of concern at hazardous waste sites. The acute MRL was based on human eye and nasal irritation in the occupational study by Hollingsworth et al. (1956). The NOAEL was 15 ppm, and the LOAEL was 30 ppm, the highest level in which odor could be detected without causing sensory irritation. An intraspecies UF = 10 was applied, resulting in an acute MRL of 2 ppm (rounded up from 1.5 ppm).

A chronic MRL was also developed by ATSDR (2006), based on increased incidence of moderate and marked (combined) eosinophilic globules in nasal epithelium of female rats in the Aiso et al. (2005b) study. A $BMCL_{10}$ of 9.51 ppm was determined by BMC modeling and used as the POD. The POD was duration-adjusted (6 hours/ 24 hours × 5 days/7 days) to continuous exposure to 1.70 ppm. This was followed by multiplying by the HEC = 0.16 for the extrathoracic region to generate a value of 0.27

ppm. A total UF = 30 was applied (3x for interspecies UF, and 10x for the intraspecies UF), resulting in a chronic MRL of 0.01 ppm.

8.5 Evidence for Differential Sensitivity of Children

1,4-DCB was identified by CARB as a Toxic Air Contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007). Under Health and Safety Code Section 39669.5, OEHHA establishes and maintains a list of TACs that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as Reference Exposure Levels for TACs are developed.

The Acute REL is based on developmental effects in rodent offspring, primarily decreased viability and decreased body weight resulting from 1,4-DCB exposure during gestation. Maternal body weight was also reduced at concentrations that caused the effects in offspring. However, OEHHA and US EPA (1991) do not assume developmental effects at maternally toxic doses result only from maternal toxicity because the results may indicate both are sensitive to the same exposure level.

Numerous population studies have suggested exposure to 1,4-DCB (as the urinary 2,5-DCP metabolite) is associated with various effects on infants and children (Phillipat et al., 2012; Buckley et al., 2018; Twum and Wei, 2011; Buttke et al., 2012; Wolff et al., 2015; Wei and Zhu, 2016c; Wolff et al., 2017. Biomonitoring surveys in pregnant women have observed associations between increased levels of the 1,4-DCB urinary metabolite, 2,5-DCP, and low birth weight of infants, as well as increased odds for respiratory and allergic outcomes. Biomonitoring surveys in children and adolescents have observed earlier onset of puberty in girls, increasing prevalence of obesity, and altered thyroid function that is associated with higher 2,5-DCP levels in their urine, implicating 1,4-DCB as an endocrine disrupting chemical. However, causal relationships between 1,4-DCB exposure and associations with reported health conditions in population surveys are inherently difficult to establish (e.g., exposure based on a single urine sample, exposure to multiple pollutants, and misclassification of self-reported data). The acute, 8-hour and chronic RELs included UFs to account for these potential increased sensitivity in children due to the potential for 1,4-DCB to cause developmental effects and changes in hormonal function in children and adolescents.

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