2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO DICHLOROBENZENES IN THE UNITED STATES

Dichlorobenzenes (DCBs) are chlorinated aromatic compounds that have three isomeric forms. 1,2-DCB is a colorless to pale yellow liquid used primarily as a precursor for 3,4-dichloroaniline herbicides. 1,3-DCB is a colorless liquid used in the production of various herbicides, insecticides, pharmaceuticals, and dyes. 1,4-DCB, the most commercially important dichlorobenzene isomer, is a volatile colorless to white crystalline material with a mothball-like, penetrating odor. It is used as a deodorant for restrooms, for moth control, and in the production of polyphenylene sulfide (PPS) resin.

DCBs are not known to occur naturally in the environment. The primary sources of 1,4-DCB of industrial or commercial origin in the environment are releases from space deodorants and moth repellants into the atmosphere. 1,4-DCB might also be released into water through waste water streams and landfill leachate and to soil through sewage sludge application, disposal of industrial waste, and atmospheric deposition. 1,2- and 1,3-DCBs are expected to be released to the environment during their use in herbicide production or during the use of other products containing these isomers. 1,2-DCB is produced in large quantities as a by-product during the production of 1,4-DCB and can be released into the environment during the disposal of unused supplies.

1,2-, 1,3-, and 1,4-DCB have similar physical and chemical properties, and consequently are expected to have similar environmental fates. DCBs will exist predominantly in the vapor-phase in the atmosphere. They are degraded in the atmosphere by reaction with hydroxyl radicals, with atmospheric lifetimes (theoretically calculated) of about 1 month. The detection of these chemicals in rainwater suggests that atmospheric removal via washout is possible. Depending on soil type, DCBs are expected to be moderately mobile in soil and to volatilize from surface water and soil surfaces to the atmosphere. Volatilization, sorption, biodegradation, and bioaccumulation are likely to be competing processes, with the dominant fate being determined by local environmental conditions.

DCB concentrations in soil, water, and food are generally low in comparison to concentrations in air, indicating that exposure of the general population to DCBs is predominantly by inhalation. Individuals are more likely to be exposed to 1,4-DCB than to the other isomers due to the widespread use of the

1,4-isomer in deodorant and moth repellent products. Measured DCB concentrations in ambient outdoor air generally range from 0.01 to 0.1 ppb for 1,2-DCB, from 0.001 to 0.1 ppb for 1,3-DCB, and from 0.01 to 1 ppb for 1,4-DCB. The average daily adult intakes of 1,2-, 1,3-, and 1,4-DCB from ambient air have been estimated to be about 1.8, 0.8, and 35 μg/day, respectively. The heavy use of products containing 1,4-DCB in homes and other buildings has resulted in higher concentrations of this substance in indoor air compared to concentrations in outdoor air. Measured 1,4-DCB concentrations in indoor air generally range from 0.1 ppb to 100 ppb. Indoor inhalation exposure to 1,2- or 1,3-DCB is not expected to be important since these substances are not used in household and consumer products to the extent of 1,4-DCB. 1,2- and 1,4-DCB have been detected in adipose tissue at concentrations ranging from <0.1 to 38 ppb and from 0.2 to 500 ppb, respectively. 1,4-DCB has been detected in blood samples at concentrations ranging from below 0.04 to 45 ppb, while measured 1,2-DCB concentrations in blood are below 3 ppb.

Children can be exposed to DCBs prenatally, as indicated by the detection of all three isomers in placenta samples, as well as through breast feeding. 1,2-DCB concentrations measured in whole human milk range from 3 to 29 ppb. 1,3- and 1,4-DCB were detected together in whole human milk with mean and maximum concentrations of 6 and 75 ppb, respectively. These isomers were detected in milkfat samples at a mean concentration of 161 ppb and a maximum concentration of 4,180 ppb. 1,2-, 1,3-, and 1,4-DCB measured separately in whole human milk samples had concentrations of 9, <5, and 25 ppb, respectively, while the milk fat of these samples contained 230 ppb of 1,2-DCB and 640 ppb of 1,4-DCB. Children and adults are perhaps at equal risk for exposure to 1,4-DCB since there is no evidence to indicate that children are likely to be exposed to lower amounts of 1,4-DCB from everyday living. While actual exposure reports are limited to a small number of case reports, available evidence suggests that children may be exposed to 1,4-DCB if they eat or play with moth balls or toilet deodorizers.

2.2 SUMMARY OF HEALTH EFFECTS

1,2-Dichlorobenzene. 1,2-DCB is quickly and extensively absorbed through both the gastrointestinal tract and the respiratory tract; studies measuring the absorption of 1,2-DCB following dermal exposure are not available. Following absorption, 1,2-DCB is distributed throughout the body, but tends to be found in greatest levels in the fat, kidney, and liver. 1,2-DCB is initially metabolized by cytochrome P-450 enzymes, specifically P4502E1, to an active epoxide followed by hydrolysis to 2,3-dichlorophenol or 3,4-dichlorophenol. The dichlorophenols may be further oxidized or, more often, be conjugated to glutathione, sulfate, or to form the glucuronide; conjugation occurs extensively, with virtually no unconjugated metabolites reported in the available studies. Metabolism is believed to occur mainly in the liver, but may occur at lower levels in other tissues, such as the kidney or lung. Elimination of 1,2-DCB from the body is rapid, with the majority of a single dose being removed within the first 75 hours postexposure; elimination occurs primarily in the urine as metabolites.

Information on health effects of 1,2-DCB in humans is essentially limited to observations of respiratory tract and eye irritation in workers chronically exposed to the vapor. The potential for inhaled 1,2-DCB to cause respiratory tract effects is also shown by the induction of nasal olfactory lesions in an acute-duration study in mice. This effect occurred at concentrations similar to or below the lowest exposure levels that caused systemic effects in rats, mice, and guinea pigs in other acute and intermediate-duration inhalation studies. No intermediate-duration studies examined the nasal cavity, indicating that a critical effect for longer-term inhalation exposures cannot be identified. The liver is the primary systemic target of toxicity in animals exposed to 1,2-DCB. Acute-, intermediate,- and chronic-duration inhalation and oral studies clearly identify the liver as a sensitive target of oral exposure, inducing increases in liver weight at low levels of exposure and histological changes such as cloudy swelling and centrilobular degeneration and necrosis at higher levels in rats and mice.

Data on the possible effects of 1,2-DCB on reproductive or developmental end points in humans are not available. Studies by both the oral and inhalation routes of exposure failed to find effects of 1,2-DCB on histology of reproductive organs or indices of reproduction in rats and mice. Similarly, limited available data suggest that inhalation and oral exposure to 1,2-DCB do not significantly affect prenatal development in rats or rabbits.

Data on the possible carcinogenic effects of 1,2-DCB in humans are not available. Exposure to 1,2-DCB by the oral route has not been shown to cause an increase in tumor formation following lifetime exposure in rats or mice. The potential carcinogenic effects of 1,2-DCB by other routes of exposure have not been evaluated. EPA determined that 1,2-DCB is not classifiable as to human carcinogenicity and categorized it in cancer weight-of-evidence Group D. The International Agency for Research on Cancer (IARC) similarly determined that 1,2-DCB is not classifiable as to carcinogenicity to humans (Group 3).

A more detailed discussion of the hepatic and respiratory effects associated with 1,2-DCB exposure follows. The reader is referred to Section 2.2, Discussion of Health Effects by Route of Exposure, for additional information on these and other health effects.

Hepatic Effects. Data on the hepatic effects of 1,2-DCB in exposed humans are not available for any exposure route. The liver is the primary target in animals orally exposed to 1,2-DCB, generally resulting in centrilobular damage in acute- and subchronic-duration studies. A single exposure to 1,500 mg/kg in rats caused lethal central necrosis. In rats exposed to 455 mg/kg/day for 15 days, severe liver damage, characterized by intense necrosis and fatty changes and porphyria, were reported. Similarly, rats exposed to 300 mg/kg/day for 10 days showed hepatic necrosis of slight severity and increased serum alanine aminotransferase (ALT). However, an acute (14-day) study by the National Toxicology Program showed no hepatic effects in male or female rats given doses as high as 500 or 1,000 mg/kg/day for 14 consecutive days. The inconsistency between these findings might be due to a small number of animals in the14-day study and a low incidence and severity of lesions in the 10-day study. Centrilobular liver effects similar to those reported in the acute studies were found in several intermediate-duration studies in rats and mice, occurring in rats exposed to 188 mg/kg/day for 138 doses, rats exposed to 400 mg/kg/day for 90 days, rats exposed to 250 mg/kg/day or greater for 13 weeks, and mice exposed to 250 mg/kg/day for 13 weeks. A chronic study in rats and mice found no nonneoplastic liver effects in either sex of either species, even at exposures up to 120 mg/kg/day, suggesting that the nonneoplastic hepatic effects of 1,2-DCB may have a threshold, which might fall between 120 and 188 mg/kg/day.

Respiratory Tract Effects. Periodic industrial hygiene surveys and medical examinations were conducted in a plant where an unreported number of men were exposed to 1,2-DCB at an average level of 15 ppm (range, 1–44 ppm) for an unreported duration; no nasal or eye irritation was attributable to exposure. Additionally, the study author noted that the researchers detected 1,2-DCB odor at a concentration of 50 ppm without eye or nasal irritation during repeated vapor inhalation experiments on animals. An earlier source reported that occupational exposure to 100 ppm of 1,2-DCB caused irritation of the eyes and respiratory passages of exposed humans. Data on the effects of 1,2-DCB on the respiratory tract in humans following oral or dermal exposure are not available.

In male mice exposed to 1,2-DCB in mean concentrations of 0, 64, or 163 ppm for 6 hours/day, 5 days/week for 4, 9, or 14 days, histopathologic lesions were observed in the olfactory epithelium of the nasal cavity at ≥64 ppm. The olfactory epithelial lesions were graded as very severe following the 4-day exposure and moderate after the 14 day exposure, indicating to the study authors that repair may occur despite continued exposure. The more severe cases were characterized by a complete loss of olfactory epithelium, which left only partially denuded basement membrane. No histological alterations were observed in the respiratory epithelium of the nasal cavity, or in the trachea or lungs. No effects on respiratory tract tissues were reported in intermediate- or chronic-duration inhalation studies in animals; however, in most cases, evaluation of nasal tissues was not conducted.

1,3-Dichlorobenzene. Data on the absorption of 1,3-DCB in humans and animals are not available for any route of exposure; however, absorption of the compound can be inferred from studies that have detected 1,3-DCB or metabolites in the breast milk, blood, and fat of humans and in the bile and urine of exposed animals. Distribution is believed to be similar to the other DCB isomers, but data demonstrating this are not currently available. Similar to the other DCB isomers, 1,3-DCB is initially metabolized by cytochrome P 450 enzymes, followed by extensive conjugation, primarily to glutathione. 1,3-DCB is eliminated mainly in the urine, similar to the other DCB isomers.

Studies on the toxic effects of 1,3-DCB in humans are not available. No studies evaluating the toxicity of 1,3-DCB following dermal or inhalation exposure in animals were located. Information on the oral toxicity of 1,3-DCB in animals is available from one 90-day systemic toxicity study and one developmental toxicity study. The intermediate-duration study found effects in the thyroid, pituitary, and liver of rats, with thyroid lesions occurring at dose levels lower than those inducing pituitary and liver effects. The information on the developmental toxicity study of 1,3-DCB is from a gavage study reported without details as an abstract, which reported no treatment-related effects on prenatal development in rats. Reproductive function and carcinogenicity have not been evaluated in humans or animals exposed to 1,3-DCB. EPA determined that 1,3-DCB is not classifiable as to human carcinogenicity and categorized it in cancer weight-of-evidence Group D. IARC similarly determined that 1,3-DCB is not classifiable as to carcinogenicity to humans (Group 3).

A more detailed discussion of the endocrine and hepatic effects associated with 1,3-DCB exposure follows. The reader is referred to Section 2.2, Discussion of Health Effects by Route of Exposure, for additional information on these and other health effects.

Endocrine Effects. In a 90-day study in rats given 0, 9, 37, 147, or 588 mg/kg/day, the most sensitive reported effects were on the pituitary and thyroid glands. Histologically, depletion of colloid density in the thyroid, characterized by decreased follicular size with scant colloid and follicles lined by cells that were cuboidal to columnar, was increased in a dose-related manner in males exposed to ≥9 mg/kg/day, and in females exposed to ≥37 mg/kg/day. The pituitary glands of males exposed to 1,3-DCB showed cytoplasmic vacuolization of the pars distalis in all exposed groups, but the incidence was statistically significant only in animals exposed to ≥147 mg/kg/day. Increases in serum cholesterol in males at

≥9 mg/kg/day and females at ≥37 mg/kg/day, and serum calcium in both sexes at ≥37 mg/kg/day were also believed by the authors to be related to effects on endocrine end points, possibly reflecting a disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus, and/or other endocrine organs.

Hepatic Effects. In male and female rats exposed by gavage to up to 735 mg/kg/day for 10 days, hepatic effects included significantly increased relative liver weight in males at ≥147 mg/kg/day and females at ≥368 mg/kg/day, and altered histopathology at ≥368 mg/kg/day in both sexes. The main hepatic histological change was dose-related centrolobular hepatocellular degeneration, characterized by varying degrees of cytoplasmic vacuolization and swelling with intact membranes. Other hepatic alterations included hepatocellular necrosis that was sporadically noted in animals exposed to ≥147 mg/kg/day; this change was usually minimal to mild, and tended to increase in incidence and severity in males in a dose-related manner. In a 90-day study of 1,3-DCB toxicity, rats of both sexes were exposed by gavage to up to 588 mg/kg/day. Relative liver weights were increased in both sexes at ≥147 mg/kg/day. Dose-related increases in histological lesions, including inflammation, hepatocellular alterations, and hepatocellular necrosis were reported at doses of ≥147 mg/kg/day. Other statistically significant liver-associated effects included significantly increased serum aspartate aminotransferase (AST) levels (90–100% higher than controls) in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day, but whether these changes were due to an effect on the liver or an endocrine effect is not clear. Serum lactate dehydrogenase (LDH) levels were also reduced in males at ≥9 mg/kg/day, but the biological significance of a decrease in liver enzymes is unclear.

1,4-Dichlorobenzene. Following inhalation or oral exposure, absorption of 1,4-DCB is rapid and complete. Data on the absorption of 1,4-DCB following dermal exposure are not available; however, absorption is believed to be very low, based on a very high (>6 g/kg) dermal LD_{50} for 1,4-DCB in rats, and on a lack of systemic effects in humans who held solid 1,4-DCB in their hands. Similar to the other dichlorobenzene isomers, 1,4-DCB is distributed throughout the body, but tends to be found in greatest levels in fat, liver, and kidney. Metabolism of 1,4-DCB is similar to that of 1,2-DCB, with an initial oxidation to an epoxide, followed by hydrolysis to 2,5-dichlorophenol. Extensive phase II metabolism occurs subsequently, with eliminated metabolites found mainly as the sulfate, glucuronide, or mercapturic acid. 1,4-DCB is eliminated almost exclusively in the urine, primarily as conjugates of 2,5-dichlorophenol.

Information on the health effects of 1,4-DCB in humans is available from limited observations in exposed workers and case reports. Workers who were chronically exposed to 1,4-DCB vapor experienced irritation of the nose and eyes and case reports of people who inhaled or ingested 1,4-DCB suggest that the liver, nervous system, and hematopoietic system are systemic targets in humans. The available limited information on these systemic effects in humans is consistent with findings in animals exposed to 1,4-DCB.

The acute, intermediate,- and chronic-duration toxicity of 1,4-DCB in animals has been evaluated in a number of studies, predominantly in rats and mice. The respiratory tract is a target of inhaled 1,4-DCB as shown by histopathological changes in the lungs of acutely exposed rats and guinea pigs and nasal olfactory epithelium of chronically exposed rats and mice. Liver and kidney effects are the best studied and most consistently observed effects of inhalation and oral exposure. There is a general pattern in which increased liver weight and hepatocellular hypertrophy are predominant effects at exposure levels below those inducing more serious histopathological changes in the liver (e.g., congestion, fatty degeneration, focal necrosis) and clinical signs of toxicity in the respiratory tract (e.g., nose and eye irritation following inhalation exposure) and nervous system (e.g., tremors and salivation). Exposure of male rats to 1,4-DCB, but not female rats or either sex of other species, causes development of renal lesions that have been shown to be the result of interaction with the protein $\alpha_{2\mu}$-globulin, a mechanism specific to male rats and not relevant to humans. There are a few reports of effects on the hematologic system, adrenal gland, and thyroid, but these occurred at inhalation or oral exposure levels similar to or higher than those causing liver and kidney effects. Chronic inhalation exposure to 1,4-DCB induced nasal olfactory epithelial lesions in rats at concentrations below those causing liver effects.

Data on the effects of 1,4-DCB on reproductive end points in humans are not available. Oral or inhalation exposure to 1,4-DCB has not been demonstrated to produce treatment-related adverse changes in reproductive tissue histology or on reproductive end points in animals. Two-generation inhalation and oral studies in rats found that 1,4-DCB did not affect reproductive performance but induced postnatal toxicity in F_1 and F_2 offspring, including reductions in survival on day 4, body weight gain, and neurobehavioral performance at doses similar to or lower than those inducing liver effects in intermediate-duration systemic toxicity studies. No teratogenic effects were induced in rats by inhalation or oral exposure to 1,4-DCB, although indications of fetotoxicity (e.g., extra ribs) occurred at levels that were maternally toxic.

1,4-DCB is carcinogenic in animals following chronic inhalation and oral exposure. Inhalation and oral lifetime studies found liver tumors in male and female mice but not in rats of either sex. Chronic oral exposure also induced renal tubular cell adenocarcinomas in male rats, but these appear to be associated with male rat-specific $\alpha_{2\mu}$-globulin nephropathy and not relevant to carcinogenicity in humans. IARC determined that 1,4-DCB is possibly carcinogenic to humans (Group 2B). The Department of Health and Human Services (DHHS) concluded that 1,4-DCB is reasonably anticipated to be a human carcinogen.

A more detailed discussion of the hepatic, respiratory, developmental, and carcinogenic effects associated with 1,4-DCB exposure follows. The reader is referred to Section 2.2, Discussion of Health Effects by Route of Exposure, for additional information on these effects and other health effects.

Hepatic Effects. In two human fatalities believed to be caused by 1,4-DCB inhalation, the subjects died of massive hepatic necrosis; the exposure concentrations are not known. A 3 year-old child who had been playing with crystals containing 1,4-DCB for 4–5 days was jaundiced with pale mucous membranes, indicative of liver damage.

Many animal studies by both the oral and inhalation routes have confirmed the liver as a sensitive target for 1,4-DCB toxicity. Inhaled exposure concentrations of 158–211 ppm, at exposure durations from 2 weeks to 7 months, resulted in increased liver weights, cloudy swelling of the liver, and, at higher exposure levels, centrilobular hypercellular hypertrophy and necrosis. Exposure to 270 ppm for 13 weeks caused increased liver weight in rats and mice and hepatocellular hypertrophy and increased serum enzymes in mice. Exposure to 538 ppm for 10 weeks, and throughout mating and gestation for females, resulted in hepatocellular hypertrophy and increased liver weights in both the parental (F_0) generation and the F_1 generation offspring. In chronic inhalation studies in rats and mice, no effects were seen in either sex of either species at 75 ppm, but at 300 ppm, histological changes in the lung were seen in male mice, but not in female mice or in either sex of rats. Acute oral studies have demonstrated hepatic effects (increased liver weight) at concentrations as low as 300 mg/kg in rats, with higher concentrations resulting in increased liver cell proliferation and vacuolated and/or basophilic cytoplasm of centrilobular cells. Similar hepatic effects occurred in mice orally exposed to 300 mg/kg/day for 1 week. In rats exposed to 1,4-DCB for 13 weeks, increased relative liver weight was seen at ≥75 mg/kg/day, with centrilobular hypertrophy present at 300 mg/kg/day (Lake et al. 1997), and necrosis reported at 1,200 mg/kg/day (NTP 1987); oral studies in mice have reported similar effects (NTP 1987). A study of 1,4-DCB in male and female Beagle dogs found that oral exposure to 50 or 75 mg/kg/day caused increased serum levels of liver enzymes, increased liver weights, hepatocellular hypertrophy, pigment deposition, and hepatic portal inflammation after 6–12 months. In the only chronic-duration (2-year) oral study of 1,4-DCB toxicity, no effects were seen in either sex of rats exposed to up to 300 mg/kg/day, while both sexes of mice showed significant, dose-related increases in hepatocellular degeneration, starting at 300 mg/kg/day.

Respiratory Tract Effects. A case of pulmonary granulomatosis was reported to have occurred in a 53-year-old woman who for 12–15 years had been inhaling 1,4-DCB crystals that were scattered on a weekly basis on the carpets and furniture of her home. A lung biopsy revealed the presence of 1,4-DCB crystals with the surrounding lung parenchyma being distorted by fibrosis, thickening of the alveolar walls, and marked infiltrates of lymphocytes and mononuclear phagocytes. These effects are most likely related to the physical interaction of 1,4-DCB crystals (or any crystals when inhaled) with lung tissue, rather than to chemical toxicity. A health survey of 58 men occupationally exposed to 1,4-DCB for 8 hours/day, 5 days/week for 8 months to 25 years (average, 4.75 years) found the odor to be faint at 15–30 ppm and strong at 30–60 ppm, with painful irritation of the nose and eyes usually occurring at concentrations ranging from 80 to 160 ppm. At levels >160 ppm, the air was considered not breathable for unacclimated persons.

An evaluation of 953 adult participants in the Third National Health and Nutrition Examination Survey of the general U.S. population found statistically significant inverse associations between blood levels of 1,4-DCB and two measures of pulmonary function. When compared with subjects in the lowest decile of 1,4-DCB blood concentration (0.10 ppb), subjects in the highest decile (>4.40 ppb) had decrements of -153 mL in forced expiratory volume in 1 second (FEV1) and -346 mL/second in maximum mid-expiratory flow rate (MMEFR). There were no significant associations with forced vital capacity (FVC) or peak expiratory flow rate (PEFR). Although it is unclear whether the observed decrements in FEV1 and MMEFR are biologically meaningful, and other studies investigating effects of 1,4-DCB on lung function are not available, the findings suggest that exposure to 1,4-DCB may possibly contribute to decreases in lung function.

Pulmonary effects (interstitial edema, congestion, and alveolar hemorrhage) were observed in rats and guinea pigs following intermittent exposure to 175 ppm of 1,4-DCB for 16 days. The experimental design and report of this study have a number of deficiencies, such that the observations provide only qualitative evidence of exposure-related acute respiratory effects. Support for the respiratory tract as a target for inhaled 1,4-DCB in animals is provided by the induction of nasal lesions in rats and mice chronically exposed to 1,4-DCB for 6 hours/day, 5 days/week for 2 years. An increased incidence of histological changes of the nasal olfactory epithelium occurred in female rats exposed to 75 or 300 ppm, and male rats and female mice exposed to 300 ppm. In rats treated with 1,200 or 1,500 mg/kg/day or greater by gavage for 13 weeks, epithelial necrosis of the nasal turbinates was reported; similar effects were not seen in mice exposed by gavage to up to 1,800 mg/kg/day, or in rats or mice exposed by gavage for 2 years to up to 600 mg/kg/day.

Developmental Effects. A 21-year-old woman who had eaten 1–2 blocks of 1,4-DCB toilet freshener per week for the first 38 weeks of pregnancy gave birth to an apparently normal child. In a 2-generation study of the effects of inhaled 1,4-DCB on reproduction and development, the number of pups that died during the perinatal period was increased, and the body weights at postnatal day 0 and 28 were significantly decreased, in animals exposed to 538 ppm; exposures to 66 or 211 ppm had no effect on developmental end points. In rabbits exposed to 300 ppm, but not those exposed to 800 ppm, there was a significant increase in the number of resorptions and the percentages of resorbed implantations per litter; the fact that the effect did not occur in the rabbits exposed to the higher exposure level suggests that it was not treatment-related. A 2-generation oral study in rats found toxicity in the offspring at doses ≥90 mg/kg/day; effects included reduced birth weight in F_1 pups, increased mortality on postnatal day 4 in F_1 and F_2 pups, clinical manifestations of dry and scaly skin (until approximately postnatal day 7) in F_1 and F_2 pups, and reduced neurobehavioral performance (draw-up reflex evaluated at weaning) in F_2 pups. No exposure-related changes occurred at 30 mg/kg/day. Other evaluations of developmental effects of 1,4-DCB following oral exposure have been negative.

Cancer. Data on the carcinogenic effects of 1,4-DCB in humans are not available. 1,4-DCB has been shown to be carcinogenic in chronic animal studies by both the inhalation and oral routes. Following lifetime inhalation exposure, a dose-related increase in hepatocellular adenomas and carcinomas was observed in mice of both sexes, whereas incidences of liver or other tumors were not increased in rats. Following lifetime oral exposure, hepatic tumors (hepatocellular adenomas and carcinomas and histiocytic sarcomas) were increased in mice of both sexes, but not in either sex of rats. The oral bioassay also found that the male rats exposed to 1,4-DCB developed renal tubular cell adenocarcinomas, but these are believed to be the result of interaction with $\alpha_{2\mu}$-globulin, a renal protein not present in humans. Data on the possible carcinogenic effects of 1,4-DCB following dermal exposure are not available.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for dichlorobenzenes. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1994k), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

1,2-Dichlorobenzene

Acute-Duration Exposure. No MRL was derived for acute-duration inhalation exposure to 1,2-DCB due to insufficient data. No information was located regarding the acute inhalation toxicity of 1,2-DCB in humans. The nasal cavity was a target of acute inhalation in animals as shown by a study in which male mice were exposed to 64 or 163 ppm of 1,2-DCB for 6 hours/day, 5 days/week for 4, 9, or 14 days (Zissu 1995). Histological examinations of the upper and lower respiratory tracts found that nasal olfactory epithelial lesions occurred at both levels of exposure. The nasal lesions were graded as very severe following the 4 day exposure and moderate after the 14 day exposure, suggesting to the study authors that some tissue repair might have occurred despite continued exposure. The more severe cases were characterized by a complete loss of olfactory epithelium, which left only the partially denuded basement membrane. No histological alterations were observed in the respiratory epithelium of the nasal cavity, or in the trachea or lungs. Nonrespiratory tissues were not evaluated in this study.

Acute systemic effects of inhaled 1,2-DCB include histopathology in the liver (marked centrilobular necrosis) and kidneys (cloudy swelling of tubular epithelium) of rats exposed to 977 ppm for 1 hour (Hollingsworth et al. 1958), but not to 539 ppm for 3 or 6.5 hours (Hollingsworth et al. 1958) or 322 ppm for 6 hours/day for 10 days (DuPont 1982). Maternal body weight gain was decreased in rats and rabbits that were exposed to 100, 200, or 400 ppm of 1,2-DCB for 6 hours/day on days 6–15 (rats) or 6–18 (rabbits) of gestation (Hayes et al. 1985). No prenatal developmental toxicity was observed in the rabbits, although skeletal variations (delayed ossification of cervical vertebral centra) occurred in fetuses of rats at 400 ppm, indicating that developmental effects occurred in rats at concentrations that also caused maternal toxicity. Based on these findings, a lowest-observed-adverse-effect level (LOAEL) of 100 ppm for systemic toxicity and 400 ppm for developmental toxicity are identified.

The nasal histopathology findings in mice show that the upper respiratory tract is a sensitive target for acute inhalation exposure to 1,2-DCB, as serious olfactory lesions occurred at exposure concentrations below those that caused systemic or developmental effects in rats and rabbits. The 64 ppm LOAEL for severe nasal olfactory lesions precludes derivation of an acute inhalation MRL for 1,2-DCB because: (1) a no-observed-adverse-effect level (NOAEL) for nasal lesions was not determined, (2) no other animal studies tested exposure levels below 100 ppm or evaluated the nasal cavity, and (3) it is not ATSDR's practice to derive MRLs based on serious LOAELs.

Intermediate-Duration Exposure. No intermediate-duration inhalation MRL was derived for 1,2-DCB due to insufficient data. Information on the toxicity of intermediate-duration inhalation exposures to 1,2-DCB is limited to the findings of a multispecies intermediate study (Hollingsworth et al. 1958) and a 2-generation reproduction study in rats (Bio/dynamics 1989). In the intermediate study, rats and guinea pigs were exposed to 49 or 93 ppm for 7 hours/day, 5 days/week for 6–7 months (Hollingsworth et al. 1958). Mice were similarly exposed to 49 ppm only, and rabbits and monkeys were similarly exposed to 93 ppm only, although the rabbit and monkey data are compromised by small numbers of animals (two rabbits/sex and two female monkeys). No compound-related histopathological or other changes occurred in any of the animals exposed to 49 ppm. The only remarkable findings at 93 ppm were statistically significant decreases in final body weight (8.9% less than controls) in male rats and absolute spleen weight (20% less than controls) in male guinea pigs, indicating that the NOAEL and LOAEL for systemic effects are 49 and 93 ppm, respectively. In the reproductive toxicity study, male and female rats were exposed to 50, 150, or 394 ppm of 1,2-DCB for 6 hours/day, 7 days/week for 10 weeks before mating and subsequently through the F_1 generation (Bio/dynamics 1989). $\alpha_{2\mu}$-Globulin-related renal changes were found in adult males of both generations at all levels of exposure, but these effects are specific to male rats and are not relevant to humans. Decreased body weight gain, increased absolute and relative liver weights, and centrilobular hepatocyte hypertrophy occurred in adult rats of both sexes and generations at ≥150 ppm, indicating that the NOAEL and LOAEL for systemic effects are 50 and 150 ppm. There were no effects on reproduction in either generation, indicating that the NOAEL for reproductive toxicity is 394 ppm. As discussed in the acute inhalation MRL section, a NOAEL of 200 ppm and a LOAEL of 400 ppm were found for developmental toxicity (skeletal variations) in rats (Hayes et al. 1985).

As discussed above, NOAELs of 49–50 ppm and LOAELs of 93–150 ppm are identified for systemic effects in intermediate-duration inhalation studies of 1,2-DCB in rats and guinea pigs (Bio/dynamics 1989; Hollingsworth et al. 1958). Neither of these studies evaluated possible effects in the nasal cavity, a known sensitive target of 1,2-DCB based on acute data. As indicated in the acute inhalation MRL section, 64 ppm was a serious LOAEL for nasal olfactory lesions in rats intermittently exposed to 1,2-DCB for 4–14 days (Zissu 1995). Derivation of an intermediate-duration MRL for 1,2-DCB is precluded because the 64 ppm serious LOAEL for acute exposure is lower than the available intermediate-duration LOAELs for systemic and developmental effects.

Chronic-Duration Exposure. No MRL was derived for chronic-duration inhalation exposure to 1,2-DCB due to insufficient data. The available information consists of two limited human reports. Workers who were exposed to concentrations of 1,2-DCB ranging from 1 to 44 ppm (average 15 ppm) for unreported durations did not experience eye or nasal irritation, or show any changes in standard blood and urine indices, as determined by periodic occupational health examinations (Hollingsworth et al. 1958). 1,2-DCB also did not cause eye or nasal irritation in workers exposed to approximately 50 ppm (researchers exposed during the conduct of inhalation studies in animals), although the odor was perceptible at this level (Hollingsworth et al. 1958). Occupational exposure to higher concentrations of 100 ppm 1,2-DCB was reported to be irritating to the eyes and respiratory passages (Elkins 1950). The lack of adequate exposure-response data and any additional information in these reports, as well as a lack of chronic toxicity data in animals, precludes derivation of a chronic inhalation MRL.

1,3-Dichlorobenzene

No MRLs were derived for inhalation exposure to 1,3-DCB due to a lack of acute-, intermediate-, and chronic-duration inhalation studies.

1,4-Dichlorobenzene

Acute-Duration Exposure.

- An MRL of 2 ppm has been derived for acute-duration (≤14 days) inhalation exposure to 1,4-DCB.

A limited amount of information is available on the toxicity of inhaled 1,4-DCB in humans. Case reports of people who inhaled 1,4-DCB provide indications that the liver and nervous system are systemic targets of inhalation toxicity in humans, but are limited by lack of adequate quantitative exposure information and/or verification that 1,4-DCB was the only factor associated with the effects (Cotter 1953; Miyai et al. 1988; Reygagne et al. 1992).

Observations in workers who were occupationally exposed to 1,4-DCB for 8 hours/day, 5 days/week for an average of 4.75 years (range from 8 months to 25 years) provide information relevant to acute inhalation exposures. The odor was found to be faint at 15–30 ppm and strong at 30–60 ppm. 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. Concentrations above 160 ppm caused severe irritation and were considered intolerable to people not adapted to it. The odor and irritation effects are considered to be good acute warning properties that are expected prevent excessive exposures, although the industrial experience indicates that it is possible for people to become sufficiently acclimated to tolerate high concentrations of the vapor (Hollingsworth et al. 1956). Periodic occupational health examinations showed no cataracts or any other lens changes in the eyes, or effects on clinical indices (red blood cell count, total and differential white blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, blood urea nitrogen, sedimentation rate, or urinalysis) attributable to exposure.

Information on effects of acute-duration inhalation exposure to 1,4-DCB in animals is available from short-term systemic toxicity studies in rats and guinea pigs (Hollingsworth et al. 1956), a male reproduction study rats (Anderson and Hodge 1976), and developmental toxicity studies in rats and rabbits (Hayes et al. 1985; Hodge et al. 1977). In the systemic toxicity study, five rats of each sex and five guinea pigs of each sex were exposed to 173 ppm of 1,4-DCB for 7 hours/day, 5 days/week for 16 days (Hollingsworth et al. 1956). Mild histological effects of interstitial edema, congestion, and alveolar hemorrhage were observed in the lungs of male rats and female guinea pigs. The experimental design and report of this study have a number of deficiencies, such that reported observations provide only qualitative evidence of exposure-related respiratory effects. In the reproduction study (a dominant lethal test), a NOAEL of 450 ppm was identified for reproductive performance in male mice that were exposed for 6 hours/day for 5 days prior to weekly mating with unexposed females for 8 weeks (Anderson and Hodge 1976). No maternal or developmental toxicity occurred in rats that were exposed to 75–500 ppm for 6 hours/day on days 6–15 of gestation (Hodge et al. 1977), indicating that the highest NOAEL for reproductive effects in rats is 500 ppm. A developmental study in which rabbits were exposed to 100–800 ppm for 6 hours/day on gestation days 6–18 found evidence of fetotoxicity (a minor variation of the circulatory system) only at 800 ppm, which was also maternally toxic as shown by body weight loss early in gestation (Hayes et al. 1985), indicating that 800 ppm is a LOAEL for maternal and developmental effects in rabbits.

The lung is the target of concern for inhaled 1,4-DCB in rats and guinea pigs acutely exposed to 173 ppm (Hollingsworth et al. 1956) because the only effects observed in the acute reproductive and developmental studies were indications of maternal and fetotoxicity in rabbits at a much higher levels of 800 ppm (Hayes et al. 1985). Support for the respiratory tract as a sensitive target for 1,4-DCB vapor in animals is provided by the induction of nasal lesions in rats intermittently exposed to levels as low as 75 ppm for 104 weeks in the study used to derive the chronic inhalation MRL for 1,4-DCB (Aiso et al. 2005b; Japan Bioassay Research Center 1995). Additionally, the animal data are consistent with the human experience indicating that occupational exposure to 1,4-DCB causes painful nose and eye irritation in the range of 50–160 ppm (Hollingsworth et al. 1956). The current Threshold Limit Value-Time Weighted Average (TLV-TWA) for 1,4-DCB of 10 ppm, which is intended to minimize the potential for eye irritation in exposed workers (ACGIH 2001), is largely based on the human findings of Hollingsworth et al. (1956).

As discussed above, eye and nose irritation are critical effects of acute and longer-term inhalation exposures to 1,4-DCB in humans. Because odor detection is a warning property expected to prevent irritation caused by 1,4 DCB (Hollingsworth et al. 1956), the highest level at which an odor was detected that was simultaneously without irritant effects, 30 ppm, was designated a minimal LOAEL for irritation for the purposes of derivation of the MRL; the 15 ppm level was therefore designated a NOAEL for irritant effects. Using the NOAEL of 15 ppm for eye and nose irritation in humans, and applying a total uncertainty factor of 10 (for individual variability), an MRL of 2 ppm was derived for acute inhalation exposure to 1,4-DCB.

Intermediate-Duration Exposure.

- An MRL of 0.2 ppm has been derived for intermediate-duration (15–364 days) inhalation exposure to 1,4-DCB.

A limited amount of information is available on the intermediate-duration toxicity of inhaled 1,4-DCB in humans. Case reports of people who inhaled 1,4-DCB over periods of months provide indications that the liver and nervous system are systemic targets of inhalation toxicity in humans, but are limited by lack of adequate quantitative exposure information and/or verification that 1,4-DCB was the only factor associated with the effects (Cotter 1953; Miyai et al. 1988; Reygagne et al. 1992).

Information on effects of intermediate-duration inhalation exposure to 1,4-DCB in animals is available from 4–7-month toxicity studies in rats, mice, guinea pigs, rabbits, and monkeys (Hollingsworth et al. 1956), a 13-week toxicity study in rats and mice (Aiso et al. 2005a), and a 2-generation reproductive/ developmental toxicity study in rats (Tyl and Neeper-Bradley 1989). These studies show that hepatic effects increase in severity with increasing level of exposure, ranging from increased liver weight at low levels to degenerative and necrotic changes at higher concentrations, and identify the liver as the most sensitive target of intermediate-duration inhalation of 1,4-DCB. The lowest reliable hepatic effect levels are identified in the 13-week and 2-generation studies, as discussed below.

In the 13-week study, groups of 10 male and 10 female F344 rats and 10 male and 10 female BDF_1 mice were chamber-exposed to 1,4-DCB vapor (>99.9% pure) at concentrations of 0, 25, 55, 120, 270, or 600 ppm for 6 hours/day, 5 days/week for 13 weeks (Aiso et al. 2005a). End points evaluated during the study included clinical signs (daily) and body weight and food consumption (weekly). End points evaluated at the end of the 13-week exposure period included hematology (red blood cells [RBC], hemoglobin [Hb], Hematocrit [Hct], mean corpuscular volume [MCV], mean corpuscular hemogloblin [MCH]), blood biochemistry (total protein, albumin, total cholesterol, triglyceride, phospholipid, AST, ALT, alkaline phosphatase, blood urea nitrogen [BUN], creatine), organ weights, and histopathology. The histological examinations were comprehensive and included the nasal cavity, in accordance with OECD test guidelines for a 90-day inhalation study (Aiso 2005; OECD 1981).

There were no exposure-related effects on survival, clinical signs, or body weight gain in the rats (Aiso et al. 2005a). Hematological changes suggestive of microcytic anemia occurred in male rats, including significantly decreased RBC count and hemoglobin concentration at ≥120 ppm, hematocrit at ≥270 ppm, and MCV and MCH at 600 ppm. Serum biochemical changes included significant increases in total protein in both sexes at 600 ppm, albumin in females at ≥270 ppm and males at 600 ppm, and total cholesterol and phospholipid in males at ≥270 ppm and females at 600 ppm, and significant decreases in triglycerides in males at 600 ppm, AST in both sexes at 600 ppm, and ALT and AP in males at ≥270 ppm. The biological significance of decreases in serum levels of liver enzymes is unclear. Organ weight changes included >10% increases in absolute and relative weights of liver in males at ≥270 ppm and females at 600 ppm, kidneys in males at ≥270 ppm, and spleen in males at 600 ppm. Histological effects included significantly increased incidences of centrilobular hepatocellular hypertrophy in the liver in male rats at 600 ppm and kidney lesions indicative of $\alpha_{2\mu}$-globulin nephropathy in male rats at ≥270 ppm. There were no histopathological changes in hematopoietic tissues, suggesting that the anemia in the male rats was secondary to $\alpha_{2\mu}$-globulin nephropathy-related effects on erythropoietin synthesis in the renal tubules.

There were no exposure-related effects on survival, clinical signs, or body weight gain in the mice (Aiso et al. 2005a). Organ weight changes in the mice included >10% increases in liver weight in males at ≥270 ppm (relative) and 600 ppm (absolute) and females at 600 ppm (absolute and relative); relative liver weights were 9.7, 9.7, 10.1, 23.9, and 62.6% higher than controls in the low- to high-dose males. There were no significant hematological changes in either sex. Serum ALT levels were significantly increased in males at ≥270 ppm (18.2, 9.1, 18.2, 54.5 and 164% higher than controls in the low- to high-dose groups). Other serum biochemical changes included significant increases in ALT in females at 600 ppm, AST in males at 600 ppm, and total cholesterol and total protein in both sexes at 600 ppm. Histological examinations showed significantly increased incidences of centrilobular hepatocellular hypertrophy in male mice at ≥270 ppm and female mice at 600 ppm; incidences in the control to high dose males were 0/10, 0/10, 0/10, 0/10, 10/10, and 10/10. Affected hepatocytes were characterized by cell enlargement, varying nuclear size and shape, and coarse chromatin and inclusion bodies in the nucleus; the severity of these lesions was rated as slight at 270 ppm (males) and moderate at 600 ppm (both sexes). The moderate hepatocellular hypertrophy in the 600 ppm male mice was accompanied by single cell necrosis (1/10) and focal liver necrosis (2/10).

The lowest effect level in the 13-week study (Aiso et al. 2005a) study is 270 ppm based on the kidney and hematological effects in male rats and liver effects in rats and mice. The kidney and hematological effects are consistent with $\alpha_{2\mu}$-globulin nephropathy, which is specific to male rats and not relevant to humans. The mice were more sensitive to the liver effects of 1,4-DCB than the rats because the only hepatic change in the 270 ppm rats was increased liver weight, whereas hepatocellular hypertrophy and increased serum ALT occurred in addition to increased liver weight in the 270 ppm mice. Additionally, at the next highest tested level of 600 ppm, the mice had nuclear changes and evidence of necrosis in the hypertrophic hepatocytes, and increased serum AST as well as ALT, whereas none of these indicators of hepatocellular damage occurred in the rats. Based on increased relative liver weight (>10%) in both species and histological and serum enzyme changes in the mice, this study identified a NOAEL of 120 ppm and a LOAEL of 270 ppm for hepatic effects.

In the two-generation study, groups of 28 Sprague-Dawley rats of each sex were exposed to actual mean 1,4-DCB concentrations of 0, 66, 211, and 538 ppm (Tyl and Neeper-Bradley 1989). Additional groups of 10 females were similarly exposed for 10 weeks in a satellite study. The animals in the main study were paired within groups for a 3 week mating period to produce the F_1 generation. Main study males that did not successfully mate in the first 10 days of the mating period were paired with the satellite females for 10 days. Main study females that did not successfully mate during the first 10 days of the mating period were paired with proven males for the remaining 11 days of the mating period. Exposures of the main study F_0 females were continued throughout the mating period and the first 19 days of gestation, discontinued from gestation day 20 through postnatal day 4, and then resumed until sacrifice at weaning on postnatal day 28. Exposures of the satellite F_0 females were continued through mating until sacrifice on gestation day 15. Exposures of the F_0 males continued until sacrificed at the end of the study and satellite mating periods. Groups of 28 F_1 weanlings/sex and satellite groups of 10 F_1 female weanlings were exposed for 11 weeks and mated as described above to produce the F_2 generation. Additionally, 20 F_1 weanlings/sex from the control and high exposure groups served as recovery animals that were observed without exposure for 5 weeks prior to sacrifice. Complete necropsies were performed on all F_0 and F_1 adult (parental) animals, F_1 recovery animals, F_1 weanlings not used in the rest of the study, and F_2 weanlings, and histology was evaluated in the F_0 and F_1 parental animals. Histological examinations were conducted on the liver and kidneys in all groups and on selected other tissues (pituitary, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and tissues with gross lesions) in the control and high-exposure groups. The kidney evaluation included examination for the presence of $\alpha_{2\mu}$-globulin droplets. Additional end points evaluated in the parental generations included clinical observations, mortality, body weight, and food consumption. Mating and fertility indices were determined for F_0 and F_1 males and females, and gestational, live birth, postnatal survival (4, 7, 14, 21, and 28 days), and lactation indices were determined for the F_1 and F_2 litters.

No effects on reproductive parameters in either generation were reported, although systemic toxicity occurred at all dose levels in F_0 and F_1 adult rats (Tyl and Neeper-Bradley 1989). Hyaline droplet nephropathy was found in F_0 and F_1 adult males at ≥66 ppm. Manifestations of this male rat-specific renal syndrome included $\alpha_{2\mu}$-globulin accumulation and increased kidney weights at ≥66 ppm, and other characteristic histological changes at 538 ppm. Body weights and weight gains were significantly reduced in F_0 and F_1 adult males and F_1 adult females during the pre-breed exposure periods at 538 ppm. Absolute liver weights were increased in F_0 males by 6, 16, and 38% in the 66, 211, and 538 ppm groups, respectively; the differences were statistically significantly different from control in the 211 and 538 ppm groups. In F_0 females, absolute liver weights were increased by 9 and 31% at 211 and 538 ppm, respectively, but statistical significance was achieved only at 538 ppm. Similar changes were seen in relative liver weights of the F_0 generation, with respective increases of 5, 14, and 52% in the 66, 211, and 538 ppm males and 4, 9, and 31% in the 66, 211, and 538 ppm females; all groups of treated males, and the 211 and 538 ppm female groups, were statistically significantly different from controls. Relative liver weights were also significantly increased in F_1 adult males at ≥211 ppm and in F_1 adult females at 538 ppm. Hepatocellular hypertrophy was observed in the livers of F_0 and F_1 males and females at 538 ppm; no hepatic histological changes were induced at the lower exposure concentrations. Other effects also occurred in the F_0 and F_1 males and females at 538 ppm, indicating that there was a consistent pattern of adult toxicity at the high exposure level, including reduced food consumption and increased incidences of clinical signs (e.g., tremors, unkempt appearance, urine stains, salivation, and nasal and ocular discharges); these effects only sporadically occurred at 211 ppm. Other effects at 538 ppm included reduced gestational and lactational body weight gain, and postnatal toxicity, as evidenced by increased number of stillborn pups, reduced pup body weight, and reduced postnatal survival in F_1 and/or F_2 litters. This study identified: (1) a NOAEL of 66 ppm and LOAEL of 211 ppm for increased (>10% above controls) relative liver weight in adult rats, and (2) a serious LOAEL of 538 ppm for systemic toxicity (central nervous system and other clinical signs) in adult rats and developmental toxicity (increased stillbirths and perinatal mortality) in their offspring (Tyl and Neeper-Bradley 1989). The identification of increased liver weight as a critical effect of 1,4-DCB toxicity is supported by findings of increased liver weight and serum liver enzyme levels and histopathologic liver lesions following repeated oral exposure (Naylor and Stout 1996).

Benchmark dose (BMD) analysis of the male rat serum ALT data (Aiso et al. 2005a) was conducted using all appropriate continuous-variable models in the EPA Benchmark Dose Software (Version 1.3.2) and a benchmark response (BMR) of 1 standard deviation change from the control mean. None of the models provided an adequate fit to the variance, precluding the use of this data set for selecting a point of departure for deriving an MRL. Available continuous-variable models were also fit to the Tyl and Neeper-Bradley (1989) data for changes in liver weight in male rats using a BMR of 1 standard deviation.

The 2-degree polynomial model was the best fitting model, predicting a benchmark concentration (BMC_{1sd}) and $BMCL_{1sd}$ (lower 95% confidence limit on the BMC_{1sd}) of 120 and 92 ppm, respectively. A summary of the predicted BMCs and BMCL for both end points, as well as details of the BMD modeling, are presented in Appendix A.

Using the $BMCL_{1sd}$ of 92 ppm for increased liver weight in male rats and EPA (1994k) inhalation reference concentration (RfC) methodology to determine the MRL, the $BMCL_{1sd}$ of 92 ppm was duration-adjusted for intermittent exposure, as follows:

$$\begin{aligned} BMCL_{1sd\ ADJ} &= (BMCL_{1sd})\ (\text{hours}/24\ \text{hours})\ (\text{days}/7\ \text{days}) \\ &= (92\ \text{ppm})\ (6\ \text{hours}/24\ \text{hours})\ (7\ \text{days}/7\ \text{days}) \\ &= 23\ \text{ppm} \end{aligned}$$

1,4-DCB exhibited the effects outside of the respiratory tract and consequently is treated as a category 3 gas for purposes of calculating the MRL. The human equivalent concentration (HEC) for extrarespiratory effects produced by a category 3 gas is calculated by multiplying the duration-adjusted $BMCL_{1sd}$ ($BMCL_{1sd\ ADJ}$, see below) by the ratio of blood:gas partition coefficients ($H_{b/g}$) in animals and humans (EPA 1994k). $H_{b/g}$ values were not available for 1,4-DCB in rats and humans. Using a default value of 1 for the ratio of partition coefficients, the $BMCL_{1sd\ HEC}$ becomes 23 ppm:

$$\begin{aligned} BMCL_{1sd\ HEC} &= (BMCL_{1sd\ ADJ})\ \text{x}\ [(H_{b/g})_{RAT} / (H_{b/g})_{HUMAN}], \\ &= 23\ \text{ppm x}\ [1] = 23\ \text{ppm} \end{aligned}$$

The $BMCL_{1sd\ HEC}$ was divided by a total uncertainty factor of 100 to derive the MRL. This uncertainty factor is comprised of component factors of 10 for interspecies extrapolation, and 10 for human variability. Although the rat exposure concentration was adjusted to a HEC, an uncertainty factor of 10 was still applied, because HEC calculation was based on an assumption of equivalent blood-gas partition coefficients, and not on actual data. Dividing the 23 ppm $BMCL_{1sd\ HEC}$ for increased liver weight in male rats by an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for human variability) yields an MRL of 0.2 ppm for intermediate-duration inhalation exposure to 1,4-DCB.

Chronic-Duration Exposure.

- An MRL of 0.01 ppm has been derived for chronic-duration (≥365 days) inhalation exposure to 1,4-DCB.

A limited amount of information is available on the long-term toxicity of inhaled 1,4-DCB in humans. Periodic occupational health examinations of workers who were exposed to 1,4-DCB for an average of 4.75 years (range, 8 months to 25 years) showed no changes in standard blood and urine indices (Hollingsworth et al. 1956). The odor was found to be faint at 15–30 ppm and strong at 30–60 ppm. 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. Concentrations above 160 ppm caused severe irritation and were considered intolerable to people not adapted to it. Occasional examination of the eyes showed no cataracts or any other lens changes. The odor and irritation properties are considered to be fairly good warning properties that should prevent excessive exposures, although the industrial experience indicates that it is possible for people to become sufficiently acclimated to tolerate high concentrations of the vapor (Hollingsworth et al. 1956). The data from this study are inadequate for chronic MRL derivation due to poor characterization of long-term exposure levels, insufficient investigation of systemic health end points, and reporting and other study deficiencies. Although the available occupational data are insufficient for chronic MRL derivation, the nose and eye irritation findings in humans are consistent with nasal effects observed in chronically exposed animals, as discussed below.

Information on the chronic inhalation toxicity of 1,4-DCB in animals is available from two studies in rats and mice (Aiso et al. 2005b; Japan Bioassay Research Center 1995; Riley et al. 1980a, 1980b). In the Riley et al. (1980a, 1980b) studies, rats of both sexes and female mice were exposed to 75 or 500 ppm of 1,4-DCB for 5 hours/day, 5 days/week for up to 76 weeks (rats) or 57 weeks (mice), followed by 32 weeks (rats) or 18–19 weeks (mice) without exposure. There were no exposure-related histopathological changes in the nasal cavity or other tissues in either species. Liver and kidney weights were increased in rats of both sexes at 500 ppm, but the toxicological significance is questionable due to the negative histopathology findings and the lack of related clinical chemistry effects. Evaluation of the mouse data is limited by reporting insufficiencies in the available summary of the study.

In the other chronic study (Aiso et al. 2005b; Japan Bioassay Research Center 1995), groups of 50 male and female F344/DuCrj rats and 50 male and female Crj:BDF$_1$ mice were exposed to 1,4-DCB in target concentrations of 0, 20, 75, or 300 ppm for 6 hours/day, 5 days/week for 104 weeks. Study end points included clinical signs and mortality, body weight (weekly for the first 13 weeks, and subsequently every 4 weeks), and hematology, blood biochemistry, and urinalysis indices (evaluated at end of study). Selected organ weight measurements (liver, kidneys, heart, lungs, spleen, adrenal, brain, testis, and ovary) and comprehensive gross pathology and histology evaluations were performed on all animals at the end of the study or at time of unscheduled death. No interim pathology examinations were performed. As summarized below, this study identifies a NOAEL of 20 ppm and a LOAEL of 75 ppm for dose-related eosinophilic changes in the olfactory epithelium in female rats.

For the rats, the actual mean chamber concentrations were 0, 19.8, 74.8, or 298.4 ppm over the duration of the study (Aiso et al. 2005b; Japan Bioassay Research Center 1995). The number of rats surviving to scheduled termination was significantly ($p<0.05$) reduced at 300 ppm in males. Survival in the male rats was noticeably lower than controls beginning at approximately study week 80, and overall survival at 0, 20, 75, and 300 ppm was 66% (33/50), 68% (34/50), 58% (29/50), and 36% (18/50), respectively. There were no exposure-related decreases in survival in the female rats, or effects on growth or food consumption in either sex. Changes in various hematological and blood biochemical indices (mean cell volume, total cholesterol, phospholipids, blood urea nitrogen, creatinine, and calcium in males; total protein, total bilirubin, blood urea nitrogen, and potassium in females) occurred at 300 ppm, but a lack of numerical data and statistical analysis precludes interpretations of significance for these end points. Absolute and relative liver weights in both sexes and kidney weights in males were significantly increased at 300 ppm. Additional findings included histopathological changes in the kidneys and nasal epithelia. The kidney lesions occurred only in male rats at 300 ppm and included significantly increased incidences of mineralization of the renal papilla and in hyperplasia of the urothelium. The nasal lesions mainly included increased incidences of eosinophilic changes (globules) in the olfactory epithelium (moderate or greater severity) in males at 300 ppm and females at ≥75 ppm. Incidences of this lesion at 0, 20, 75, and 300 ppm were 1/50, 2/50, 2/50, and 7/50 in males, and 28/50, 29/50, 39/50, and 47/50 in females. The increases were statistically significant ($p\leq0.05$, Fisher's Exact Test performed by ATSDR) at ≥75 ppm in females and 300 ppm in males, and there was a trend of increasing response with increasing dose in both sexes (Cochran-Armitage test, performed by ATSDR). Other nasal lesions that were significantly increased at 300 ppm were eosinophilic globules in the respiratory epithelium (11/50, 10/50, 14/50, 38/50) and respiratory metaplasia in the nasal gland (5/50, 4/50, 4/50, 33/50) in females at 300 ppm. Kidney lesions were increased only in male rats at 300 ppm and included significantly increased incidences of mineralization of the renal papilla (0/50, 1/50, 0/50, 41/50) and in hyperplasia of the urothelium (7/50, 8/50, 13/50, 32/50).

For the mice, the actual mean chamber concentrations were 0, 19.9, 74.8, or 298.3 ppm over the duration of the study. Survival was significantly reduced in male mice at 300 ppm (due to an increase in liver tumor deaths), but comparable to controls in the females. Terminal body weight was significantly reduced at 300 ppm in males (11.5% less than controls, beginning at study week 80). Changes in various hematological and blood biochemical indices (total cholesterol, serum glutamic oxaloacetic transaminase [SGOT], serum glutamic pyruvic transaminase [SGPT], lactic dehydrogenase [LDH], and alkaline phosphatase [AP] in both sexes; platelet numbers, total protein, albumin, total cholesterol, blood urea nitrogen, and calcium in females) occurred at 300 ppm (Japan Bioassay Research Center 1995), but a lack of reported numerical data and results of statistical analysis precludes interpretation of these end points. Absolute and relative liver and kidney weights in both sexes were significantly increased at 300 ppm. Additional findings included histopathological changes in the nasal cavity, liver, and testes. The nasal lesions included significantly increased incidences of respiratory metaplasia in the nasal gland (moderate severity) in males at 75 ppm (9/49, 12/49, 18/50, 11/49) and olfactory epithelium (slight severity) in males at 75 ppm (23/49, 30/49, 37/50, 22/49) and females at 300 ppm (7/50, 6/50, 2/49, 20/50); the effects in the males were not dose-related (i.e., incidences were increased at 75 ppm but not at 300 ppm). The incidence of centrilobular hepatocellular hypertrophy was significantly increased in male mice at 300 ppm (0/49, 0/49, 0/50, 34/49). Incidences of liver tumors were also increased at 300 ppm; these included hepatocellular carcinoma in males (12/49, 17/49, 16/50, 38/49) and females (2/50, 4/50, 2/49, 41/50), hepatocellular adenoma in females (2/50, 10/50, 6/49, 20/50), hepatoblastoma in males (0/49, 2/49, 0/50, 8/49) and females (0/50, 0/50, 0/49, 6/50), and histiocytic sarcoma in males (0/49, 3/49, 1/50, 6/49). Testicular mineralization was significantly increased in males at ≥75 ppm (27/49, 35/49, 42/50, 41/49) (Japan Bioassay Research Center 1995). The testicular mineralization was not considered to be a toxicologically significant effect (Aiso 2006) because (1) no signs of testicular toxicity were observed in mice exposed for 13 weeks (Aiso et al. 2005a), and (2) it was confined to the testicular capsules and testicular 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 (Aiso 2006).

The results of this study indicate that moderate or severe eosinophilic changes in the nasal olfactory epithelium in female rats is the most sensitive toxic effect in the most sensitive species and sex. The NOAEL and LOAEL for these nasal lesions are 19.8 and 74.8 ppm, respectively. To derive a point of departure for MRL derivation, BMD analysis was conducted using the incidences of the nasal lesions (moderate or greater severity) in the female rats. Data for other end points were not modeled because the effects occurred at higher concentrations (nasal lesions and hepatocellular hypertrophy in mice, kidney lesions in rats) or were not toxicologically significant (testicular mineralization in mice). All dichotomous models in the Benchmark Dose Software (version 1.3.2) were fit to the female rat nasal lesion incidence data. All models provided adequate fits to the data, and the quantal linear model provided the best fit to the data. Using a BMR level of 10% extra risk above the control incidence, the quantal linear model resulted in a benchmark concentration (BMC_{10}) of 14.08 ppm and lower 95%

confidence limit ($BMCL_{10}$) of 9.51 ppm. A summary of the predicted BMCs and BMCLs, as well as details of the BMD modeling, are presented in Appendix A.

Using the $BMCL_{10}$ value of 9.51 ppm for increased incidences of nasal lesions in female rats and EPA (1994k) inhalation RfC methodology to determine the MRL, the $BMCL_{10}$ was duration-adjusted for intermittent exposure, as follows:

$$\begin{aligned} BMCL_{10\ ADJ} &= (BMCL_{10})\ (\text{hours}/24\ \text{hours})\ (\text{days}/7\ \text{days}) \\ &= (9.51\ \text{ppm})\ (6\ \text{hours}/24\ \text{hours})\ (5\ \text{days}/7\ \text{days}) \\ &= 1.70\ \text{ppm} \end{aligned}$$

For the nasal olfactory epithelium changes in female rats, 1,4-DCB was treated as a category 1 gas with effects in the extrathoracic region for purposes of calculating the HEC. Using EPA (1988, 1994b) reference values, the regional gas deposition ratio was calculated as follows (EPA 1994a):

$$\begin{aligned} RGDR_{ET} &= [(V_E/SA_{ET})_A/(V_E/SA_{ET})_H] \\ &= (0.24\ m^3/\text{day}/15cm^2)/(20\ m^3/\text{day}/200cm^2) \\ &= 0.16 \end{aligned}$$

where: $RGDR_{ET}$ = regional gas deposition ratio in the extrathoracic region

V_E = minute volume in rats $(V_E)_A$ or humans $(V_E)_H$

SA_{ET} = extrathoracic surface area in rats $(SA_{ET})_A$ or humans $(SA_{ET})_H$

The HEC was calculated by multiplying the rat $BMCL_{10\ ADJ}$ by the $RGDR_{ET}$ to yield a $BMCL_{10\ HEC}$ of 0.27 ppm, as follows:

$$\begin{aligned} BMCL_{10\ HEC} &= BMCL_{10\ ADJ} \times RGDR_{ET} \\ &= 1.70\ \text{ppm} \times 0.16 \\ &= 0.27\ \text{ppm} \end{aligned}$$

The $BMCL_{10\ HEC}$ of 0.27 ppm for nasal effects in rats was divided by a total uncertainty factor of 30 to calculate the MRL. This uncertainty factor is comprised of component factors of 3 for interspecies extrapolation and 10 for human variability. A 3-fold uncertainty factor was used instead of a default 10-fold factor to extrapolate from rats to humans, because the dosimetry adjustment (i.e., calculation of the human equivalent exposure for time and concentration [$NOAEL_{HEC}$]) addresses one of the two areas of uncertainty encompassed in an interspecies extrapolation factor. The dosimetric adjustment addresses the pharmacokinetic component of the extrapolation factor, but the pharmaco-dynamic area of uncertainty remains as a partial factor for interspecies uncertainty. Dividing the 0.27 ppm $NOAEL_{10\ HEC}$ by the uncertainty factor of 30 yields an MRL of 0.01 ppm for chronic-duration inhalation exposure to 1,4-DCB.

Oral MRLs

1,2-Dichlorobenzene

Acute-Duration Exposure.

- An MRL of 0.7 mg/kg/day has been derived for acute-duration (≤14 days) oral exposure to 1,2-DCB.

Information on effects of acute oral exposure to sublethal doses of 1,2-DCB consists of findings in three systemic toxicity studies in rats and mice and one developmental toxicity study in rats (NTP 1985; Rimington and Ziegler 1963; Robinson et al. 1991; Ruddick et al. 1983). These studies administered the compound by gavage and collectively identify the liver as the most sensitive target. Severe liver damage, characterized by intense necrosis and fatty changes as well as porphyria, occurred in rats administered 455 mg/kg/day for 15 consecutive days (Rimington and Ziegler 1963). Rats that were exposed to 300 mg/kg/day for 10 consecutive days had hepatic effects that included necrosis and increased serum ALT (Robinson et al. 1991). Hepatocellular degeneration and necrosis occurred in mice that were exposed to 250 or 500 mg/kg/day for 14 consecutive days (NTP 1985). The 15-day rat and 14-day mouse studies are limited by small numbers of animals (3–5 per dose) and lack of a NOAEL due a single dose level (Rimington and Ziegler 1963) or lack of histopathology evaluations at doses lower than the LOAEL (NTP 1985). The 10-day study (Robinson et al. 1991) is the most appropriate basis for MRL derivation because it is well designed, included four dose levels, and provides dose-response data for several hepatic end points.

In the Robinson et al. (1991) study, groups of 10 male and 10 female Sprague-Dawley rats were treated with 1,2-DCB in corn oil by gavage at doses of 0, 37.5, 75, 150, or 300 mg/kg/day for 10 consecutive days. The doses were selected on the basis of a reported rat oral LD_{50} of 500 mg/kg. End points evaluated during the study included clinical signs, body weight, and food and water consumption. Evaluations at the end of the exposure period included hematology (five indices), serum chemistry (nine indices including aspartate AST, ALT, LDH, cholesterol, blood urea nitrogen, and creatinine), and selected organ weights (brain, liver, spleen, lungs, thymus, kidneys, adrenal glands, heart, and testes or ovaries). Histological examinations were performed on various tissues including liver, kidneys, urinary bladder, heart, skin, muscle, bone, respiratory tract (nasal cavity with turbinates, lungs), nervous system (brain, sciatic nerve), immunological (spleen, thymus, lymph nodes), gastrointestinal (duodenum, ileum, jejunum, salivary gland, colon, cecum, rectum), endocrine (adrenal glands, pancreas), and reproductive (testes, seminal vesicles, prostate, ovaries) in the high-dose and control groups. Target organs identified in the high-dose group were also histologically evaluated at the lower dose levels.

No clinical signs or effects on survival were observed (Robinson et al. 1991). Body weight gain was significantly reduced in the male rats at 300 mg/kg/day (final body weights were 10.9% lower than controls), but not in females, and there were no exposure-related changes in food consumption in either sex. Statistically significant changes in organ weights predominantly occurred at 300 mg/kg/day, including significantly decreased absolute spleen weight in both sexes and decreased absolute heart, kidney, thymus, and testes weights in males. Liver weight (absolute and relative) was significantly increased in females at ≥150 mg/kg/day and males at 300 mg/kg/day; compared to controls in the low- to high-dose females, absolute liver weights were 1.8, 9.0, 20.5, and 29.0% increased and relative liver weights were 6.8, 7.6, 21.7, and 34.5% increased. Clinical chemistry findings included significantly increased serum ALT in both sexes at 300 mg/kg/day and serum phosphorus in females at ≥150 mg/kg/day. Serum cholesterol was significantly increased in females at ≥37.5 mg/kg/day, but the toxicological significance is unclear because the values were similar at all dose levels and showed no dose-response. Histopathological findings were limited to the liver and included necrosis that was slight in severity and significantly (p=0.04) increased in males at 300 mg/kg/day (4/10 compared to 0/10 in controls); incidences in the other dose groups were not reported, although the study authors indicated that target organs in the high-dose groups were histologically evaluated at the lower dose levels. Incidences of other hepatic lesions were not significantly increased, but included inflammation (characterized by lymphocyte and macrophage infiltrates) and degeneration of hepatocytes (characterized by varying degrees of fibrillar or vacuolated cytoplasm and swelling with intact cell membranes). This study identified a NOAEL of 75 mg/kg/day and a LOAEL of 150 mg/kg/day for increased liver weight (>10%) in female rats, as well as a LOAEL of 300 mg/kg/day for liver necrosis in male rats.

To derive a point of departure for MRL derivation, BMD dose analysis was conducted using the rat absolute liver weight data. The liver lesion data were not subjected to BMD analysis because incidences of liver necrosis were only reported for control and high-dose rats. Serum liver enzyme (ALT, AST, LDH) data were not subjected to BMD analysis because a statistically significant increase was noted only for serum ALT in the high-dose group of male rats and the magnitude of the increase (50% higher than the control serum ALT level) is not considered to be adverse. All continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the absolute liver weight data from male and female rats. One standard deviation increase from the control mean value was selected as the BMR in the absence of a biological rationale for using an alternative BMR. A summary of the predicted BMDs and BMDLs, as well as details of the BMD modeling, are presented in Appendix A. The linear model was determined to the best-fitting model for the liver weight data in male rats (provided a BMD_{1sd} of 249.04 mg/kg/day and a $BMDL_{1sd}$ of 158.55 mg/kg/day) and female rats (provided a BMD_{1sd} of 84.67 mg/kg/day and a $BMDL_{1sd}$ of 67.73 mg/kg/day). Among the best-fitting model results, the lowest $BMDL_{1sd}$ of 67.73 mg/kg/day was selected as the point of departure for deriving the MRL. The $BMDL_{1sd}$ of 67.73 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive an MRL of 0.7 mg/kg/day for acute-duration oral exposure to 1,2-DCB.

Intermediate-Duration Exposure.

- An MRL of 0.6 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to 1,2-DCB.

Information on effects of intermediate-duration oral exposure to 1,2-DCB is available from three intermediate studies in rats and mice identifying the liver as the most sensitive target of toxicity (Hollingsworth et al. 1958; NTP 1985; Robinson et al. 1991). Incidences of degenerative liver lesions were significantly increased in rats exposed to 250–500 mg/kg/day for ≥13 weeks (Hollingsworth et al. 1958; NTP 1985; Robinson et al. 1991) and mice exposed to 250 mg/kg/day for 13 weeks (NTP 1985). Necrotic lesions occurred in several rats at 125 mg/kg/day (1/10 males, 3/10 females), but the increase was not statistically significant (NTP 1985). Other hepatic findings in rats exposed to lower doses (125–188 mg/kg/day for ≥13 weeks) included increases in relative liver weight and serum levels of ALT, cholesterol, serum protein, and decreases in serum triglycerides. Increased serum ALT is an inconsistent finding because it was induced in rats exposed to ≥100 mg/kg/day for 90 days (Robinson et al. 1991), but not in rats exposed to ≥125 mg/kg/day for 13 weeks (NTP 1985). Additionally, the increase in serum ALT was not dose-related and serum levels of other liver-associated enzymes were not increased in either the Robinson et al. (1991) study (AST, LDH, and AP) or the NTP (1985) study (AP and GGTP). The lowest LOAEL is 125 mg/kg/day, which is a minimal LOAEL for increased liver weight in rats in the NTP (1985) study.

In the NTP (1985) study, groups of 10 male and 10 female F344 rats and 10 male and 10 female $B6C3F_1$ mice were administered 1,2-DCB in doses of 0, 30, 60, 125, 250, or 500 mg/kg/day for

5 days/week for 13 weeks. Histology examinations of the liver were limited to the control and three highest dose groups. Degenerative lesions were significantly ($p \leq 0.05$) increased in both species at ≥250 mg/kg/day. Changes in the rats included necrosis of individual hepatocytes at ≥250 mg/kg/day and centrilobular degeneration at 500 mg/kg/day; total incidences of these lesions at 0, 125, 250, and 500 mg/kg/day were 0/10, 1/10, 4/9, and 8/10 in males, and 0/10, 3/10, 5/10, and 7/8 in females. Relative liver weights were significantly increased at 125, 250, and 500 mg/kg/day in the males (8, 17, and 45% higher than controls) and females (8, 15, and 30%); increased relative liver weights were not seen at lower doses in either sex. There were no increases in serum levels of liver enzymes [ALT, AP, or GGPT] at any dose in either sex. Serum cholesterol was significantly increased in males at ≥30 mg/kg/day (50.0, 17.6, 26.5, 70.6, and 109% higher than controls in the low to high dose groups; not significant at 60 mg/kg/day) and females at ≥125 mg/kg/day (12.2, 12.2, 32.6, 26.5, and 51.0%). Although increases in serum cholesterol were observed at doses as low as 30 mg/kg/day, the toxicological significance is unclear because there was no clear dose-response unless the increase at 30 mg/kg/day is considered to be outlying. Urinary concentrations of uroporphyrin and coproporphyrin were 3–5 times higher than controls in the 500 mg/kg/day males and females, but this increase was not considered indicative of porphyria because total porphyrin concentration in the liver was not altered at any dose level and no pigmentation indicative of porphyria was observed by ultraviolet light at necropsy. The 60 and 125 mg/kg/day doses are the NOAEL and LOAEL, respectively, for hepatic effects in rats based on the increases in liver weight in both sexes.

In the mice, no compound-related histopathological changes were observed in either sex at 0 and 125 mg/kg/day or in females at 250 mg/kg/day. Lesions that were significantly increased included necrosis of individual hepatocytes, hepatocellular degeneration and/or pigment deposition in 4/10 males at 250 mg/kg/day, and centrilobular necrosis, necrosis of individual hepatocytes, and/or hepatocellular degeneration in 9/10 males and 9/10 females at 500 mg/kg/day. Relative liver weights were significantly increased at 500 mg/kg/day in both sexes, but there were no exposure-related changes in serum levels of ALT, AP, or GGPT in either sex at any dose (no other clinical chemistry indices were examined in the mice). Based on the liver lesion data, the NOAEL and LOAEL in mice are 125 and 250 mg/kg/day, respectively.

To derive a point of departure for MRL derivation, BMD analysis was conducted using liver lesion and liver weight data from the NTP (1985) study. Dichotomous models available in the EPA Benchmark Dose Software (Version 1.3.2) were fit to data for incidences of liver lesions (single cell necrosis, centrilobular necrosis, and/or hepatocellular degeneration) in male and female rats (combined) and male mice. Because there were no apparent differences in sensitivity to 1,2-DCB among the male and female rats, the liver lesion data were combined to increase the statistical power for BMD analysis. For each data set, BMDs and their lower 95% confidence limits (BMDLs) were calculated using a BMR of 10% extra risk. All available models provided adequate fit to liver lesion data for male and female rats combined. The best-fitting model was the quantal quadratic model, which provided a BMD_{10} of 108.71 mg/kg/day and a $BMDL_{10}$ of 92.08 mg/kg/day. The log-probit model was determined to be the best-fitting model for the male mouse incidence data and provided a BMD_{10} of 176.05 mg/kg/day and $BMDL_{10}$ of 114.58 mg/kg/day. Continuous variable models in the EPA Benchmark Dose Software were fit to the relative liver weight data for male and female rats using a BMR of 1 standard deviation from the control mean. Adequate fits were not obtained for the male rat liver weight data, but the linear model was the best-fitting model for the female data, resulting in a BMD_{1sd} of 108.15 mg/kg/day and a $BMDL_{1sd}$ of 89.27 mg/kg/day. A summary of the predicted BMDs and BMDLs for both end points, as well as details of the BMD modeling, are presented in Appendix A.

The $BMDL_{1sd}$ of 89.27 mg/kg/day from the best-fitting modeling results of the female rat relative liver weight data is lower than the $BMDL_{10}$ of 92.08 mg/kg/day from the best-fitting modeling results of liver lesion incidences in the male and female rats combined and the $BMDL_{10}$ of 114.58 mg/kg/day from the best-fitting model results of liver lesion incidences in the male mice. Therefore, the $BMDL_{1sd}$ of 89.27 mg/kg/day for increased relative liver weight in the female rats was selected as the point of departure for the MRL. The $BMDL_{1sd}$ of 89.27 mg/kg/day was adjusted for intermittent experimental exposure (5 days/7 days) to give a duration-adjusted $BMDL_{1sd}$ of 63.76 mg/kg/day, and divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive an intermediate-duration oral MRL of 0.6 mg/kg/day for 1,2-DCB.

Chronic-Duration Exposure.

- An MRL of 0.3 mg/kg/day has been derived for chronic-duration (≥365 days) oral exposure to 1,2-DCB.

One chronic oral toxicity study of 1,2-DCB is available. In this study groups of F344/N rats (50/sex/group) and $B6C3F_1$ mice (50/sex/group) were administered 1,2-DCB in corn oil by gavage in doses of 0, 60, or 120 mg/kg/day for 5 days/week for 103 weeks (NTP 1985). Evaluations included clinical signs, body weight, and necropsy and histology on all animals. Organ weight and clinical chemistry indices were not assessed. The only exposure-related effect in either species was a significantly increased incidence of renal tubular regeneration in the male mice. This lesion showed a dose-related trend, and was statistically significantly elevated in high-dose animals, but not in low-dose animals. The NOAEL for the lesion was therefore 60 mg/kg/day, and the LOAEL was 120 mg/kg/day.

To derive a point of departure for MRL derivation, BMD analysis was conducted using the kidney lesion incidence data. All dichotomous models in the Benchmark Dose Software (version 1.3.2) were fit to the male mouse incidence data for renal tubule regeneration. A 10% extra risk above the control incidence was selected as the BMR in the absence of a biological rationale for using an alternative BMR. A summary of the predicted BMDs and BMDLs, as well as details of the BMD modeling, are presented in Appendix A. The logistic model was the best-fitting model, resulting in a BMD_{10} of 62.96 mg/kg/day and a $BMDL_{10}$ of 43.04 mg/kg/day. The $BMDL_{10}$ 43.04 mg/kg/day was adjusted for intermittent experimental exposure (5 days/7 days) to give a duration-adjusted $BMDL_{10}$ of 30.74 mg/kg/day, and divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive a chronic-duration oral MRL of 0.3 mg/kg/day for 1,2-DCB.

1,3-Dichlorobenzene

Acute-Duration Exposure.

- An MRL of 0.4 mg/kg/day has been derived for acute-duration (≤14 days) oral exposure to 1,3-DCB.

The acute oral database for 1,3-DCB consists of one short-term toxicity study in which groups of 10 male and 10 female Sprague Dawley rats were administered gavage doses of 0, 37, 147, 368, or 735 mg/kg/day in corn oil for 10 consecutive days (McCauley et al. 1995). End points evaluated during the study included clinical signs, survival, body weight, and food and water consumption. At the end of the study, blood was collected for hematology and serum chemistry analyses (erythrocytes, leukocytes, hemoglobin, hematocrit, mean corpuscular volume, glucose, BUN, creatinine, AP, AST, ALT, cholesterol, LDH, and calcium levels), and selected organs were weighed (brain, liver, spleen, lungs with lower half of trachea, thymus, kidneys, adrenal glands, heart, and gonads). Gross pathology was evaluated in all animals, and comprehensive histological examinations were performed in the high dose and control groups; histology in the lower dose groups was limited to the liver. Inflammatory and degenerative lesions were graded on a relative scale from one to four depending on the severity (minimal, mild, moderate, or marked).

No compound-related deaths or overt clinical signs were observed (McCauley et al. 1995). Body weight was significantly reduced in both sexes at 735 mg/kg/day (20 and 13% lower than controls in males and females, respectively). Food consumption was significantly decreased at 735 mg/kg/day in males (12%, normalized by body weight), and water consumption was significantly increased (8–13%) in females at ≥735 mg/kg/day. The hematological evaluation showed 8% decreased MCV in females at 735 mg/kg/day. The clinical chemistry analyses showed statistically significant changes in several indices, but serum cholesterol was the only end point that had values that exceeded the reference range. Serum cholesterol was significantly increased in females at 368 and 735 mg/kg/day (94 and 63% higher than controls, respectively), as well as in males at 368 and 735 mg/kg/day (79 and 84% higher than controls, respectively). Relative liver weight was significantly increased in males at ≥147 mg/kg/day and females at ≥368 mg/kg/day; increases in the males were 9.1, 31.3, 50.63, and 32.5% higher than controls in the low- to high-dose groups. Other significant changes in relative organ weight included decreased spleen weight in females at ≥368 mg/kg/day and males at 735 mg/kg/day, decreased thymus weight in both sexes at 735 mg/kg/day, and decreased testes weight in males at 735 mg/kg/day. Absolute organ weights were not reported. Histological changes primarily occurred in the liver, particularly centrilobular hepatocellular degeneration at ≥368 mg/kg/day. This lesion was characterized by varying degrees of cytoplasmic vacuolization and swelling with intact membranes, and occurred in the 368 and 735 mg/kg/day groups in 2/10 and 9/10 males, respectively, and 6/10 and 10/10 females, respectively; incidences in the other groups were not reported but are presumed to be 0/10. Other hepatic alterations included hepatocellular necrosis that was sporadically noted in the 147, 368, and 735 mg/kg/day groups. This change was usually minimal to mild, and was reported to increase in incidence and severity in the males in a dose-related manner; however, incidences were not reported. The only other reported histological change was atrophy of the thymus, characterized by loss of normal differentiation between medulla and cortex. The thymic atrophy was observed in 2/10 males (both marked in severity) and 2/9 females (both mild in severity) at 735 mg/kg/day; this change was not observed in controls, and the other dosed groups were not examined. The 147 mg/kg/day dose is the LOAEL (minimal) for liver effects based on the >10% increase in relative liver weight in male rats. The NOAEL for increased liver weight is 37 mg/kg/day.

To derive a point of departure for MRL derivation, BMD analysis was conducted using liver effects data from the McCauley et al. (1995) study. The liver effects data modeled included incidences of hepatocellular degeneration, absolute liver weights and mean serum cholesterol levels. All dichotomous variable models available in the EPA Benchmark Dose Software (Version 1.3.2) were fit to the incidence data for hepatocellular degeneration in male and female rats using a BMR of 10% extra risk. All continuous variable models in the BMD software were fit to the mean absolute liver weight data and mean serum cholesterol level data in male and female rats using a BMR of 1 standard deviation increase above the control mean. A summary of the predicted BMDs and BMDLs for all end points, as well as details of the BMD modeling, are presented in Appendix A. The best-fitting models resulted in a $BMDL_{10}$ of 207.86 mg/kg/day for hepatocellular degeneration in male rats (log-probit model), a $BMDL_{10}$ of 159.37 mg/kg/day for hepatocellular degeneration in female rats (log-probit model), and a $BMDL_{1sd}$ of 36.32 mg/kg/day for absolute liver weight changes in female rats (2-degree polynomial model). The lowest $BMDL_{1sd}$ of 36.32 mg/kg/day was selected as the most conservative point of departure for deriving an MRL. The $BMDL_{1sd}$ of 36.32 mg/kg/day was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive an acute-duration oral MRL of 0.4 mg/kg/day for 1,3-DCB.

Intermediate-Duration Exposure.

- An MRL of 0.02 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to 1,3-DCB.

The database for intermediate-duration oral exposure to 1,3-DCB consists of one intermediate toxicity study in which groups of 10 male and 10 female Sprague Dawley rats were administered gavage doses of 0, 9, 37, 147, or 588 mg/kg/day in corn oil for 90 consecutive days (McCauley et al. 1995). End points evaluated during the study included clinical signs and mortality, body weight, and food and water consumption. At end of the exposure period, blood was collected for hematology and serum chemistry analyses (erythrocytes, leukocytes, hemoglobin, hematocrit, mean corpuscular volume, glucose, BUN, creatinine, AP, AST, ALT, cholesterol, LDH, and calcium levels), selected organs were weighed (brain, liver, spleen, lungs with lower half of trachea, thymus, kidneys, adrenal glands, heart, and gonads), and gross pathology was assessed. Histological examinations were performed on all tissues that were examined grossly in all high-dose rats and in one-half of control rats, as well as in the liver, thyroid, and pituitary glands from all animals in the 9, 37, and 147 mg/kg/day dose groups. Inflammatory and degenerative lesions were graded on a relative scale from one to four depending on the severity (minimal, mild, moderate, or marked).

No compound-related deaths or overt clinical signs were observed (McCauley et al. 1995). Body weight was reduced in both sexes at 588 mg/kg/day (24 and 10% lower than controls in males and females, respectively). The decreased weight gain was progressive throughout the exposure period and occurred despite increased food and water consumption in the same groups. Other effects included increased relative kidney weight in males at ≥147 mg/kg/day and females at 588 mg/kg/day, but there were no renal histopathological changes in any of the exposed animals. Hematological alterations consisted of significant increases in leukocyte levels in males at 147 mg/kg/day and females at 588 mg/kg/day, and in erythrocyte levels in males at 588 mg/kg/day. As discussed below, histopathology and serum chemistry findings indicated that the thyroid, pituitary, and liver were the most sensitive targets of toxicity.

Thyroid effects included significantly ($p \leq 0.05$) increased incidences of reduced colloidal density in follicles that exceeded normal variability in male rats at ≥9 mg/kg/day and female rats at ≥37 mg/kg/day (control to high dose group incidences of 2/10, 8/10, 10/10, 8/9, and 8/8 in males, and 1/10, 5/10, 8/10, 8/10, and 8/9 in females) (McCauley et al. 1995). Depletion of colloid density in the thyroid was characterized by decreased follicular size with scant colloid and follicles lined by cells that were cuboidal to columnar. The severity of the colloid density depletion generally ranged from mild to moderate, increased with dose level, and was greater in males than females. Incidences of male rats with thyroid colloidal density depletion of moderate or marked severity were significantly increased at ≥147 mg/kg/day (0/10, 0/10, 2/10, 5/9, and 6/8).

Pituitary effects included significantly ($p \leq 0.05$) increased incidences of cytoplasmic vacuolization in the pars distalis in male rats at ≥147 mg/kg/day (2/10, 6/10, 6/10, 10/10, and 7/7). The vacuoles were variably sized, irregularly shaped, and often poorly defined, and the severity of the lesions (i.e., number of cells containing vacuoles) ranged from minimal to mild and generally increased with increasing dose level. Incidences of male rats with pituitary cytoplasmic vacuolization of moderate or marked severity were significantly increased at 588 mg/kg/day (1/10, 0/10, 2/10, 3/9, and 7/7). The pituitary lesion was reported to be similar to "castration cells" found in gonadectomized rats and considered to be an indicator of gonadal deficiency. No compound-related pituitary lesions were observed in female rats. Serum cholesterol was significantly increased in males at ≥9 mg/kg/day and in females at ≥37 mg/kg/day in a dose-related manner, and serum calcium was significantly increased in both sexes at ≥37 mg/kg/day. The investigators suggested that these serum chemistry changes might reflect a disruption of hormonal feedback mechanisms, or target organ effects on the pituitary, hypothalamus, and/or other endocrine organs.

Hepatic effects occurred in both sexes at 147 and 588 mg/kg/day, including significantly increased relative liver weight and incidences of liver lesions (McCauley et al. 1995). Absolute organ weights were not reported. Liver lesions were characterized by inflammation, hepatocellular alterations (eosinophilic homogeneous inclusions), and hepatocellular necrosis. Liver lesions that were significantly ($p \leq 0.05$) increased included hepatocellular cytoplasmic alterations of minimal to mild severity in males at ≥147 mg/kg/day (1/10, 2/10, 1/10, 6/10, and 7/9) and females at 588 mg/kg/day (0/10, 2/10, 0/10, 1/10, and 7/9), and necrotic hepatocyte foci of minimal severity at 588 mg/kg/day in both males (1/10, 2/10, 1/10, 2/10, and 5/9) and females (0/10, 0/10, 0/10, 3/10, and 5/9). Other statistically significant liver-associated effects included significantly increased serum AST levels (90–100% higher than controls) in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day. Serum cholesterol levels were significantly increased in males at ≥9 mg/kg/day and females at ≥37 mg/kg/day, but might be pituitary-related, as indicated above. Serum LDH levels were reduced in males at ≥9 mg/kg/day and BUN levels were reduced in both sexes at 588 mg/kg/day, but the biological significance of decreases in these indices is unclear.

To derive a point of departure for MRL derivation, BMD analysis was conducted using data for thyroid and pituitary lesion incidences and serum AST and cholesterol levels. Continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to serum AST levels in the male rats and the serum cholesterol levels in the male and female rats using a one standard deviation change from the control mean as the BMR. Dichotomous variable models in the BMD software were fit to the incidence data for thyroid lesions (reduced follicular colloidal density) and pituitary lesions (cytoplasmic vacuolation in the pars distalis) in the male rats. A summary of the predicted BMDs and BMDLs for all of the end points, as well as details of the BMD modeling, are presented in Appendix A. None of the models provided an adequate fit for the serum AST, serum cholesterol, or thyroid lesion data. For the pituitary lesion incidence data, all of the models provided adequate fit. The probit model provided the best fit, but nearly identical fits were provided by three other models (gamma, quantal-linear, and Weibull). Because the BMD_{10} of 4.08 mg/kg/day and associated $BMDL_{10}$ of 2.10 mg/kg/day from the gamma, quantal-linear, and Weibull models are lower than those from the probit model (BMD_{10} = 7.79 mg/kg/day; $BMDL_{10}$ = 4.46 mg/kg/day), a conservative health protective approach was taken and the lower $BMDL_{10}$ of 2.10 mg/kg/day was selected as the point of departure for deriving the MRL. The $BMDL_{10}$ of 2.1 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive an MRL of 0.02 mg/kg/day for intermediate-duration oral exposure to 1,3-DCB.

Chronic-Duration Exposure.

No MRL was derived for chronic-duration oral exposure to 1,3-DCB due to a lack of chronic oral studies.

1,4-Dichlorobenzene

Acute-Duration Exposure. No acute-duration oral MRL was derived for 1,4-DCB due to insufficient data. Information on effects of non-lethal acute-duration oral exposures to 1,4-DCB is essentially limited to hepatic and renal changes of unclear toxicological significance observed in studies designed to elucidate mechanisms of liver and kidney toxicity in rats and mice. Acute liver damage, as assessed by histopathology and serum enzyme/biochemical indicators following gavage exposure, was not induced by high levels of 1,4-DCB in rat given single doses of ≤2790 mg/kg (Allis et al. 1992), rats and mice given single doses of ≤1,200 mg/kg/day (Eldridge et al. 1992), or rats and mice administered ≤300 and ≤600 mg/kg/day, respectively, 5 days/week for 1 week (Lake et al. 1997). Porphyria, manifested as increased porphyrin levels in liver and urine and suggestive of hepatic damage, was reported in rats that were orally exposed to 770 mg/kg/day for 5 days (Rimington and Ziegler 1963). Although there was no clear evidence of liver injury in acute studies, similar dose levels of 1,4-DCB are toxic following intermediate- and chronic-duration exposures.

Increased hepatocelluar proliferation, as measured by increased incorporation of bromodeoxyridine (BrdU) or [3H] thymidine into DNA-synthesizing liver cells, has been demonstrated in rats and mice at doses ≥150 mg/kg/day in a number of single dose and short-term oral studies that found no histological or other indications of overt liver damage (Eldridge et al. 1990, 1992; Hasmall et al. 1997; Lake et al. 1997; Sherman et al. 1998; Umemura et al. 1992, 1996). The induction of liver cell proliferation in the absence of manifest hepatoxicity suggests that the proliferation is a response to mitogenic stimulation rather than compensatory regeneration to cytotoxicity. Cellular proliferation and other changes have also been demonstrated in the kidney tubular epithelia of male rats, but not in female rats or mice of either sex, following short-term oral exposures to doses ≥150 mg/kg/day (Eldridge et al. 1992; Lake et al. 1997; Sherman et al. 1998; Umemura et al. 1992). The renal effects are consistent with the induction of $\alpha_{2\mu}$-globulin nephropathy in male rats by similar doses of 1,4-DCB in other acute oral studies (Charbonneau et al. 1989b; Dietrich and Swenberg 1991; Saito et al. 1996), but are not relevant to humans. Induction of hepatic microsomal xenobiotic metabolizing enzymes appears to be the most sensitive effect of acute/short-term exposure to 1,4-DCB (Elovaara 1998). For example, oral exposure to doses as low as 20 mg/kg/day for 14 days increased the activities of glucuronyl transferase, benzpyrene hydroxylase, and enzymes involved in the detoxification of O-ethyl-O-nitrophenyl phenylphosphorothionate (EPN) in rats (Carlson and Tardiff 1976). Induction of hepatic microsomal enzymes is not necessarily adverse, but does indicate that the liver is sensitive to relatively low doses of 1,4-DCB.

The toxicological significance of the hepatic microsomal enzyme changes is unclear and the information on other liver effects is insufficient to identify a reliable NOAEL or LOAEL for acute/short-term oral exposure to 1,4-DCB. The lack of adequate data on the threshold of adverse effects precludes derivation of an MRL for acute duration oral exposure.

Intermediate-Duration Exposure.

- An MRL of 0.07 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to 1,4-DCB.

Information on the systemic toxicity of intermediate-duration oral exposure to 1,4-DCB is available from a number of studies conducted in rodents, mainly rats and mice, as well as one study in dogs. Liver and kidney effects are the most consistently observed, best characterized, and most sensitive findings in these studies. The lowest observed adverse effect level is for liver toxicity in dogs, although reproductive and developmental studies in rats indicate that offspring are particularly sensitive to 1,4-DCB toxicity during the postnatal preweaning period.

Hepatic effects induced by intermediate-duration oral exposures to 1,4-DCB ranged from increased liver weight and hepatocyte enlargement to hepatocellular degeneration, lesions, necrosis, and tumors in rats, mice, rabbits, and dogs. Increases in serum levels of enzymes and alterations in other end points (e.g., serum cholesterol and triglycerides) indicative of hepatocellular damage or liver dysfunction have also been induced. Increased liver weight is the most sensitive hepatic end point in intermediate-duration studies in rats, observed at doses as low as 150 mg/kg/day for 4–13 weeks and 188 mg/kg/day for 192 days (Hollingsworth et al. 1956; Lake et al. 1997; Umemura et al. 1998). There was no indication of early liver damage in rats exposed to 150 mg/kg/day for 4 weeks using an immunohistochemical marker of centrilobular hepatocyte injury (Umemura et al. 1998), and increases in liver porphyrins in rats exposed to 50–200 mg/kg/day for 120 days were not considered to be toxicologically significant (Carlson 1977). Hepatocellular hypertrophy and decreased serum triglycerides occurred in rats exposed to ≥300 mg/kg/day for 13 weeks (Lake et al. 1997; NTP 1987). Higher dose levels of 1,4-DCB induced degenerative liver lesions in rats exposed to 376 mg/kg/day for 192 days (slight cirrhosis and focal necrosis) (Hollingsworth et al. 1956) or 1,200 mg/kg/day for 13 weeks (hepatocyte degeneration and necrosis) (NTP 1987). In mice, hepatocellular degeneration was induced at doses ≥600 mg/kg/day for 13 weeks (NTP 1987), and rabbits had cloudy swelling and minimal focal necrosis in the liver after exposure to 500 mg/kg/day for 367 days (Hollingsworth et al. 1956). Dogs are more sensitive to hepatic effects of intermediate-duration 1,4-DCB exposure than the other species because serum enzyme levels were increased following exposure to doses as low as 50 mg/kg/day for 6 months (Naylor and Stout 1996).

Renal changes, including hyaline droplet accumulation, increased kidney weights, and tubular lesions, are characteristically observed effects of subchronic and chronic oral exposure to 1,4-DCB in male rats at doses ≥75 mg/kg/day (Bomhard et al. 1988; Lake et al. 1997; NTP 1987). These findings are not considered for MRL derivation because there is a scientific consensus that they are related to the $\alpha_{2\mu}$-globulin nephropathy syndrome, which is specific to male rats and not relevant to humans. Subchronic studies in female rats found increased kidney weight, but no indications of nephrotoxic action (i.e., no histopathology or effects on urinary indices of renal function), following exposure to ≥188 mg/kg/day for 192 days or 600 mg/kg/day for 13 weeks (Bomhard et al. 1988; Hollingsworth et al. 1956).

Developmental toxicity studies provide no indications that 1,4-DCB is teratogenic in rats at oral doses as high as 1,000 mg/kg/day during gestation, although fetotoxicity occurred at maternally toxic levels ≥500 mg/kg/day (Giavini et al. 1986; Ruddick et al. 1983). Decreased maternal weight gain and increased incidences of extra ribs, a skeletal variation attributable to the maternal toxicity, occurred in rats at gestational dose levels ≥500 mg/kg/day, but not at 250 mg/kg/day (Giavini et al. 1986). In a two-generation study, reproductive and developmental toxicity were evaluated in male and female rats that were orally exposed to 30, 90, or 270 mg/kg/day of 1,4-DCB (Bornatowicz et al. 1994). No effects on mating and fertility indices were observed at any level, although toxicity occurred in the offspring at doses ≥90 mg/kg/day. Effects at ≥90 mg/kg/day included reduced birth weight in F_1 pups and increased total number of deaths from birth to postnatal day 4 in F_1 and F_2 pups, clinical manifestations of dry and scaly skin (until approximately postnatal day 7) and tail constriction with occasional partial tail loss (during postnatal days 4–21) in F_1 and F_2 pups, reduced neurobehavioral performance (draw-up reflex evaluated at weaning) in F_2 pups, and increased relative liver weight in adult F_1 males. No exposure-related changes were found at 30 mg/kg/day, indicating that this is the NOAEL for reproductive and developmental toxicity in rats.

As discussed above, liver, kidney, and perinatal developmental toxicity are main effects of concern for intermediate-duration oral exposure to 1,4-DCB in animals. The dog is the most sensitive tested species, as liver effects were induced by exposure to doses as low as 50 mg/kg/day for 6 months (Naylor and Stout 1996), which are below subchronic LOAELs of approximately 150–200 mg/kg/day for liver and kidney effects in rats and mice. The two-generation study in rats demonstrates that oral exposure to 1,4-DCB can cause perinatal developmental toxicity, including reduced birth weight and neonatal survival in F_1 and F_2 pups, at doses ≥90 mg/kg/day (Bornatowicz et al. 1994). Although this finding indicates that perinatal developmental toxicity is an additional sensitive end point for 1,4-DCB exposure, the lower 50 mg/kg/day LOAEL for liver effects in dogs (Naylor and Stout 1996) is a more appropriate basis for MRL derivation.

In the dog study, groups of five male and five female beagles were orally administered 1,4-DCB by capsule in dose levels of 0, 10, 50, or 75 mg/kg/day on 5 days/week for 1 year (Naylor and Stout 1996). Complete details on the experimental design and results of the study are provided in the section on the chronic oral MRL for 1,4-DCB. As summarized below, 6-month interim liver enzyme findings are consistent with liver enzyme, liver weight, and histopathological changes observed at 1 year. Hepatic end points evaluated at 6 months were limited to clinical chemistry indices, including serum ALT, AST, GGTP, and AP, whereas the 1-year end-of-study evaluations included liver weight and histology in addition to clinical chemistry. Effects on serum enzymes included statistically significantly increased AP in males at 50 mg/kg/day after 6 and 12 months, females at 50 mg/kg/day after 6 and 12 months, and females at 75 mg/kg/day after 6 and 12 months. Serum AP levels were not statistically significantly increased in the 75 mg/kg/day males at months 6 or 12, but only three animals were evaluated in this dose group. As detailed in the chronic MRL summary, the increases in serum AP were similar in magnitude after 6 and 12 months, ranging from 330 to 761% higher than control values. Other clinical chemistry findings included significantly increased serum ALT (75 mg/kg/day females at month 12) and GGTP (75 mg/kg/day females at months 6 and 12), and significantly decreased albumin (50 and 75 mg/kg/day in males at months 6 and 12, and 75 mg/kg/day in females at month 6). Absolute and relative liver weights were significantly increased in both sexes at 50 and 75 mg/kg/day (except absolute liver weight in 50 mg/kg/day males). Hepatic lesions included hepatocellular hypertrophy (diffuse or multifocal in all males and females at 50 and 75 mg/kg/day, and one female at 10 mg/kg/day), hepatocellular pigment deposition (two males and one female each at 50 and 75 mg/kg/day), bile duct/ductule hyperplasia (one male and one female at 75 mg/kg/day), and hepatic portal inflammation (periportal accumulation of neutrophils in one male at 50 mg/kg/day and two males at 75 mg/kg/day). The 50 mg/kg/day dose is a intermediate-duration LOAEL based on the increases in serum AP at 6 months. This serum enzyme change is a sufficient indication of intermediate-duration hepatotoxicity because the increases were similar in magnitude to those observed after 1 year and associated with increased liver weight and liver lesions; the latter effects likely developed earlier in the study but could not be detected due to the lack of organ weight and histology examinations before 1 year.

To derive a point of departure for MRL derivation, BMD analysis was conducted using the Naylor and Stout (1996) data for changes in serum AP levels in female dogs. Mean serum AP levels in the female dogs exhibited a dose-response relationship and were significantly increased in the 50 and 75 mg/kg/day groups. Although significantly increased mean serum AP levels were noted in the 50 mg/kg/day male dogs, the increase was not significant in the 75 mg/kg/day males; only three males in this dose group were available for the assessment of serum AP levels. Therefore, the male serum AP data were not modeled. Continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to serum AP data in the female dogs using a one standard deviation change from the control mean as the BMR. A summary of the predicted BMDs and BMDLs, as well as details of the BMD modeling, are presented in Appendix A. The best fit was provided by the polynomial model, which resulted in a BMD_{1sd} of 12.48 mg/kg/day and a $BMDL_{1sd}$ of 9.97 mg/kg/day. The $BMDL_{1sd}$ of 9.97 mg/kg/day was adjusted for intermittent experimental exposure (5 days/7 days) to give a duration-adjusted $BMDL_{1sd}$ of 7 mg/kg/day, and divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive an intermediate-duration oral MRL of 0.07 mg/kg/day for 1,4-DCB.

Chronic-Duration Exposure.

- An MRL of 0.07 mg/kg/day has been derived for chronic-duration (365 days or more) oral exposure to 1,4-DCB.

Information on the chronic oral effects of 1,4-DCB is available from one study each in rats, mice, rabbits, and dogs. Observed effects included nephropathy in rats (including tubular degeneration and atrophy in females) exposed to ≥150 mg/kg/day on 5 days/week for 103 weeks (NTP 1987), hepatocellular degeneration and nephropathy in mice exposed to ≥300 mg/kg/day on 5 days/week for 103 weeks (NTP 1987), and cloudy swelling and minimal focal necrosis in rabbits exposed to 500 mg/kg/day in 263 doses in 367 days (Hollingsworth et al. 1956). The lowest chronic LOAEL in these studies was 150 mg/kg/day for kidney effects in female rats (NTP 1987). Liver and kidney effects were induced in dogs at doses below the LOAELs in the other species. As summarized below, doses as low as 50 mg/kg/day for 1 year were hepatotoxic in dogs (Naylor and Stout 1996).

In the dog study, groups of five male and five female beagles were orally administered 1,4-DCB by capsule in dose levels of 0, 10, 50, or 75 mg/kg/day for 1 year (Naylor and Stout 1996). Based on the summarized design of a 4-week dose range-finding study, it is presumed that dosing was 5 days/week. The 75 mg/kg/day dose is a time-weighted average level reflecting dose decreases at the beginning of the study in response to unexpected severe toxicity. An initial high dose of 150 mg/kg/day was adjusted to 100 mg/kg/day for males during week 3, and a further decrease to 75 mg/kg/day was made for both sexes at the beginning of week 6. Both high-dose males and females were untreated during weeks 4 and 5 to allow for recovery. End points evaluated throughout the study included clinical observations (daily), body weight (weekly), and food consumption (weekly). Ophthalmoscopic examinations were performed prior to study start and just prior to study completion. Hematology (11 indices, including activated partial thromboplastin time), clinical chemistry (18 indices, including ALT, AST, GGTP, AP, and creatinine phosphokinase), and urinalysis (10 indices) were performed at month 6 and study completion (month 12). Organ weights, gross pathology, and histology were evaluated at month 12.

Mortality occurred the first 25 days of the study before dose reduction; exposure to 150 mg/kg/day caused one male dog to be sacrificed *in extremis* on day 12, one male death on day 25, and one female death on day 24 (Naylor and Stout 1996). A control male died on day 83, but all other dogs survived to the end of the study. Treatment-related clinical signs were primarily limited to severely affected high-dose dogs and the control male that died; these included hypoactivity, dehydration, decreased defecation, blood-like fecal color, emesis, emaciation, and/or pale oral mucosa. There were no significant group differences in mean body weight at the end of the study. Body weight gain was significantly reduced during the first month of the study, but recovered following dose reduction and adjustment of food availability. A mild anemia was observed at month 6 (significantly reduced red blood cells in females and HCT in males) at 75 mg/kg/day, but it resolved by the end of the study. The mild anemia correlated with histologic findings of bone marrow erythroid hyperplasia in females, and splenic excessive hematopoiesis and megakaryocyte proliferation in both sexes, indicating a compensatory response to the earlier anemia. Hepatic effects occurred at ≥50 mg/kg/day in both sexes as shown by changes in liver enzymes, increased liver weight, and histopathology. Effects on serum enzyme levels included significantly increased AP in males at 50 mg/kg/day at months 6 and 12 (731 and 620% higher than controls, respectively), females at 50 mg/kg/day at months 6 and 12 (525 and 330% higher), and females at 75 mg/kg/day at months 6 and 12 months (761 and 680% higher). Serum AP was also increased in males at 75 mg/kg/day after 6 and 12 months, but the changes were not statistically significant, possibly due to a reduced group size of 3 males at 75 mg/kg/day. Other clinical chemistry findings included significantly increased ALT in females at 75 mg/kg/day at month 12 (253% higher than controls), increased GGTP in females at

75 mg/kg/day at months 6 and 12 (131 and 161% higher), and decreased albumin in males at 50 and 75 mg/kg/day at month 6 (16 and 18% lower than controls) and females at 75 mg/kg/day at month 6 (19% lower). Absolute and relative liver weights were significantly increased (40–70% higher than controls) in both sexes at 50 and 75 mg/kg/day (except absolute liver weight in 50 mg/kg/day males). Hepatocellular hypertrophy (diffuse or multifocal) occurred in all males and females at 50 and 75 mg/kg/day and in one female at 10 mg/kg/day. The study authors (Naylor and Stout 1996) considered the hepatocellular hypertrophy (multifocal) in the single 10 mg/kg/day female dog to be an adaptive response to a xenobiotic agent rather than a direct treatment related effect. Other liver lesions considered to be treatment-related included hepatocellular pigment deposition (two males and one female each at 50 and 75 mg/kg/day), bile duct/ductule hyperplasia (one male and one female at 75 mg/kg/day), and hepatic portal inflammation (periportal accumulation of neutrophils in one male at 50 mg/kg/day and two males at 75 mg/kg/day). Kidney effects included collecting duct epithelial vacuolation in one male at 75 mg/kg/day and at all dose levels in females (one each at 10 and 50 mg/kg/day and two at 75 mg/kg/day). The renal lesion was considered to be a possible effect of treatment at ≥50 mg/kg/day, because it was accompanied by increased relative kidney weight in females at ≥50 mg/kg/day and grossly observed renal discoloration in two females at 75 mg/kg/day. The highest chronic NOAEL and lowest LOAEL are 10 and 50 mg/kg/day, respectively, based on the hepatic effects in dogs (increased liver weight, changes in liver enzymes, and histopathology).

To derive a point of departure for MRL derivation, BMD analysis was performed on the serum AP level and relative liver weight data for the female dogs. The incidences of hepatocellular hypertrophy in the females (0/5, 1/5, 5/5, and 5/5 at 0, 10, 50, and 75 mg/kg/day) and males (0/5, 0/5, 5/5, and 5/5) are inappropriate for BMD modeling due to actual or effective responses of 0% in the control and low dose groups and 100% in the higher dose groups. The response in the low-dose female dog is effectively 0% because the authors implied that the hypertrophy in this single animal was not a hepatotoxic response. The incidences of the other dog liver lesions were not subjected to BMD analysis due to the low numbers of responders and group sizes. Continuous variable models in the EPA Benchmark Dose Software (Version 1.3.2) were fit to serum AP and relative liver weight data in the female dogs using a one standard deviation change from the control mean as the BMR. A summary of the predicted BMDs and BMDLs, as well as details of the BMD modeling, are presented in Appendix A. The relative liver weight data were judged to be unsuitable for BMD analysis due to inadequate modeling of variance. The best fit for the serum AP data was provided by the polynomial model, which resulted in a BMD_{1sd} of 15.40 mg/kg/day and a $BMDL_{1sd}$ of 12.32 mg/kg/day. The $BMDL_{1sd}$ of 12.32 mg/kg/day was rounded to one significant figure (10 mg/kg/day), adjusted for intermittent experimental exposure (5 days/7 days) to give a duration-adjusted $BMDL_{1sd}$ of 7 mg/kg/day, and divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to derive a chronic-duration oral MRL of 0.07 mg/kg/day for 1,4-DCB.