4-CHLORO-1,2-BENZENEDIAMINE

CAS # 95-83-0

ORAL RISK ASSESSMENT DOCUMENT

NSF International
Ann Arbor, MI
July 2006

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EXECUTIVE SUMMARY

4-CHLORO-1,2-BENZENEDIAMINE – Oral Risk Assessment CAS # 95-83-0			
PARAMETER	**LEVEL**	**UNITS**	**DERIVED**
$BMDL_{10}$ (95% confidence limit at 10% response level)	45	mg/kg-day	From a chronic feeding study in rats
10^{-5} Cancer Risk Level	0.005	mg/kg-day	From the $BMDL_{10}$
Oral Slope Factor	0.0022	$mg/kg\text{-}day^{-1}$	From the $BMDL_{10}$
Drinking Water Unit Risk (at the 1 x 10^{-6} cancer risk level)	0.063 x 10^{-6}	$\mu g/L^{-1}$	From the oral slope factor
TAC (total allowable concentration)	0.2	mg/L	For a 70 kg adult drinking 2 L/day
SPAC (single product allowable concentration)	0.02	mg/L	From the TAC, assuming 10 potential sources of 4-chloro-1,2-benzenediamine in drinking water
STEL (short term exposure level)	Not applicable	Not applicable	The STEL is not derived for a genotoxic carcinogen

KEY STUDY	National Toxicology Program (NTP)/National Cancer Institute (NCI). 1978. Bioassay of 4-Chloro-o-phenylenediamine For Possible Carcinogenicity. DHEW Publication No. (NIH) 78-1313, U.S. Department of Health Education and Welfare, National Cancer Institute, Bethesda, MD 20014.
CRITICAL EFFECT	Combined urinary bladder tumors and precursor effects, including urinary bladder transitional cell carcinomas, papillary carcinomas, transitional cell papillomas, papillomas not otherwise specified, and urinary bladder papillomatosis in male and female rats.
UNCERTAINTY FACTORS	No uncertainty factors were applied in this risk assessment, since an oral RfD was not determined. The carcinogenic effects observed in chronic animal studies were analyzed using linear extrapolation.
TOXICITY SUMMARY	No human data were located. Hepatic focal hyperplasia and renal pyelonephritis were among the non-neoplastic effects observed in rats at dose-related increased incidences compared to controls after dietary administration of 4-chloro-1,2-benzenediamine for 78 weeks. Urinary bladder tumors and precursor effects were among the neoplastic effects observed in male and female rats. Uterine/endometrial hyperplasia was observed in female mice at an increased incidence compared to controls after chronic dietary administration of 4-chloro-1,2-benzenediamine for 78 weeks, and combined hepatocellular adenomas and carcinomas were observed in male or female mice at a statistically increased incidence compared to controls. No kinetic or metabolism studies in humans or laboratory animals were identified for 4-chloro-1,2-benzenediamine. The weight of evidence suggests that 4-chloro-1,2-benzenediamine is genotoxic *in vivo* and *in vitro*. 4-Chloro-1,2-benzenediamine caused mutations in *Salmonella typhimurium* with metabolic activation and in hepatocytes isolated from Big Blue™ mice exposed *in vivo*. 4-Chloro-1,2-benzenediamine also caused chromosomal aberrations *in vivo* in mouse bone marrow cells and produced dose-related increases in micronucleated polychromatic erythrocytes in the *in vivo* mouse bone marrow micronucleus assay. Sister chromatid exchanges were also observed *in vivo*, but the effect was not dose-related. Positive results in an *in vivo* alkaline single cell (Comet) assay were observed in the liver, but not in other organs. Unscheduled DNA synthesis was detected in an *in vitro* DNA repair assay in primary rat hepatocytes. 4-Chloro-1,2-benzenediamine was positive in an *in vitro* microscreen prophage-induction assay with metabolic activation in *Escherichia coli* for the ability to induce DNA damage. A 10^{-5} cancer risk level for 4-chloro-1,2-benzenediamine was extrapolated from the chronic feeding $BMDL_{10}$ of 45 mg/kg-day, which was based on the combined incidence of urinary bladder tumors and precursors in rats, since these tumors represented the most sensitive endpoint in laboratory animals.
CONCLUSIONS	Based on the statistically increased tumor incidences in rats and mice chronically administered 4-chloro-1,2-benzenediamine, and its positive *in vitro* and *in vivo* genotoxicity data, the weight of evidence supports that 4-chloro-1,2-benzenediamine is *likely to be carcinogenic to humans*. The drinking water action levels derived in this risk assessment are protective of public health since they were based on chronic oral data for 4-chloro-1,2-benzenediamine from the most sensitive endpoint and laboratory animal species.

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1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **4-chloro-1,2-benzenediamine** in drinking water, as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2005), or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2005). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993; U.S. EPA, 2002), which assumes that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996; U.S. EPA, 2002; WHO/IPCS, 2005) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED_{10} or $BMDL_{10}$ from benchmark dose programs) can be used (U.S. EPA, 2003a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 2003b).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70\text{ kg}] - [\text{total contribution of other sources (mg/day)}]}{2\text{ L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70\text{ kg}}{2\text{ L/day}} \times 0.2\text{ (RSC)}$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and distribution system. In the absence of source data, a default multiple source factor of 10 is used.

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This accounts for the possibility that more than one product in the water and/or its distribution system could contribute the contaminant in question to drinking water.

Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED_{10} of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to a level at or below the TAC under static conditions, or to a level at or below the SPAC under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a, U.S. EPA, 1999; U.S. EPA, 2003c; U.S. EPA, 2005a). If there is sufficient evidence to use a non-linear model, the LED_{10} or $BMDL_{10}$, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED_{10} or $BMDL_{10}$ to zero, is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10^{-6} to 10^{-4} is considered by the U.S. EPA to be safe and protective of public health (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2005) and 61 (2005), the TAC is set at the 10^{-5} risk level, and the SPAC is set at the 10^{-6} risk level. Use of a higher risk level is not ruled out, but would generally require documentation of a benefit to counteract the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2005) and 61 (2005). Application of these values to other exposure scenarios should be done with care, and with a full understanding of the derivation of the values and of the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation, and require the use of additional uncertainty factors to protect public health.

The general guidelines for this risk assessment include those from the National Research Council (NRC, 1983) and from the Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a; 1997b). Other guidelines used in the development of this assessment may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), draft final Guidelines For Carcinogen Risk Assessment (U.S. EPA, 2003c), Guidelines For Carcinogen Risk Assessment (U.S. EPA, 2005a), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998), A Review of the Reference Dose and Reference Concentration Process (U.S. EPA, 2002), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing Guidelines (U.S. EPA, 2005b).

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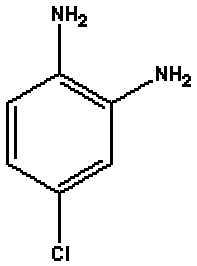
The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART)
- TOXLINE – Core and Special
- TRI (Toxics Release Inventory)
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)
- Current Contents (as requested)

The literature search for this chemical was conducted on June 22, 2004. This document includes all relevant information retrieved as a result of that search.

2.0 PHYSICAL AND CHEMICAL PROPERTIES

4-Chloro-1,2-benzenediamine is a halogenated aromatic amine with the following structure.



4-Chloro-1,2-benzenediamine has synonyms of 1,2-diamino-4-chlorobenzene, 1-chloro-3,4-diaminobenzene, 2-amino-4-chloroaniline, 3,4-diamino-1-chlorobenzene, 3,4-diaminochloro-benzene, 4-chloro-1,2-diaminobenzene, p-chloro-1,2-phenylenediamine, and 4-chloro-ortho-phenylenediamine, and 4-cl-o-pd. It has trade names of C.I. 76015 (ChemIDPlus, 2005; ChemFinder, 2005). Physical and chemical properties are listed in Table 1:

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Table 1. The physical and chemical properties of 4-chloro-1,2-benzenediamine

Property	Data	Reference
Empirical Formula	$C_6H_7ClN_2$	ChemIDPlus, 2005
CAS#	95-83-0	ChemIDPlus, 2005
Molecular Weight	142.59	Lide, 1995
Physical State and Color	Crystalline powder	NTP, 2002
Melting Point	76 °C	Lide, 1995
Boiling Point	Not located	
Density	Not located	
Vapor Pressure	Not located	
Water Solubility	<0.1 g/100 mL at 19 °C	ChemFinder, 2005
pK_a	Not located	
n-Octanol/Water Partition Coefficient (log K_{ow})	0.8 (estimated) 1.28 (experimental)	SRC, 2005
Henry's Law Constant (air/water partition)	Not located	

2.1 Organoleptic Properties

No information on the organoleptic properties of 4-chloro-1,2-benzenediamine was located.

3.0 PRODUCTION AND USE

3.1 Production

4-Chloro-1,2-benzenediamine has been produced commercially since the early 1940s (IARC, 1982). No current information on the production volume was identified, but in 1977 production of 4-chloro-1,2-benzenediamine in the United States was estimated to be 1,000 to 10,000 pounds (NTP, 2002). It is produced by the reduction of 4-chloro-1,2-dinitrobenzene (IARC, 1982).

3.2 Use

4-Chloro-1,2-benzenediamine is a curing agent for epoxy resins. It is also used as an oxidative base and as a reagent in gas chromatography (NTP, 2002), and is believed to be a chemical intermediate in the production of 5-chlorobenzotriazole (HSDB, 2005; NTP, 2002). 4-Chloro-1,2-benzenediamine has been patented for use as a hair-dye component (NTP, 2002; IARC, 1982), with a maximum concentration in the formulations of less than 1% (CIR, 1992). It is permitted in the European Communities as a hair dye oxidizing coloring agent at levels below 6% in the finished product (HSDB, 2005).

No accepted uses for 4-chloro-1,2-benzenediamine as a direct or indirect food additive were identified under Title 21 of the U.S. Code of Federal Regulations (U.S. FDA, 2005). Also, no uses for 4-chloro-1,2-benzenediamine as a direct or indirect drinking water additive have been evaluated by NSF. However, a number of non-halogenated phenylenediamine derivatives used as antioxidants and antiozonants in rubber articles intended for repeated food contact are listed in 21 CFR section 177.2600. These derivatives are frequently used in products evaluated by NSF,

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such as gaskets, o-rings, and other generally small rubber parts with the potential to contact drinking water.

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

In water, chlorobenzenediamine isomers are quantitated at NSF International using a method employing gas chromatography with electron capture or mass spectrometric detection. The detection limit is 0.5 ppb. Based on relative retention times and comparison of mass spectra to those of authentic standards, identification of the individual isomers can be accomplished.

The analysis of 4-chloro-1,2-benzenediamine in aqueous environmental samples using high performance liquid chromatography with either an ultraviolet or an electrochemical detector has been described by Riggin and Howard (1983). The water samples were extracted with methylene chloride. The electrochemical method using 0.7 Volts had a better detection limit than the ultraviolet method at 254 nanometers. Using electrochemical detection, the estimated detection limit for 4-chloro-1,2-benzenediamine was 0.6 ng of injected chemical.

4.2 Analysis in Biological Matrices

No information pertaining to the analysis of 4-chloro-1,2-benzenediamine in biological matrices was located.

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

The primary routes of potential human exposure to 4-chloro-1,2-benzenediamine are ingestion, inhalation, and dermal contact during its production or use (NTP, 2002). However, the extent of human exposure to 4-chloro-1,2-benzenediamine is unknown (IARC, 1982). Exposure could occur if accidental releases of the compound into the work environment took place. Because of its limited use in consumer products, little exposure is expected in the general population. Nevertheless, exposure could potentially occur if residues were present in some hair dyes or in products made from 5-chlorobenzotriazole (NTP, 2002).

4-Chloro-1,2-benzenediamine is not used directly in material formulations. However, benzenediamine compounds are used as ingredients in rubber additives and in some proprietary processes, and may react with available chlorine from other ingredients in a material formulation or in drinking water.

5.2 Sources of Environmental Exposure

No information was located regarding the potential for environmental exposure to chlorobenzenediamines. Due to their industrial uses, environmental exposure could occur if accidental releases into the environment took place.

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6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

No studies examining the absorption, distribution, metabolism or elimination of 4-chloro-1,2-benzenediamine in humans or laboratory animals were identified in the literature.

7.0 EFFECTS ON HUMANS

7.1 Case Reports

No case reports of exposure to 4-chloro-1,2-benzenediamine were identified in the published literature.

7.2 Epidemiological Studies

No epidemiological studies pertaining to 4-chloro-1,2-benzenediamine were identified in the published literature.

8.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

No studies assessing dermal or occular irritation or dermal sensitization were identified for 4-chloro-1,2-benzenediamine. Subchronic (eight-week) dietary administration of 1% 4-chloro-1,2-benzenediamine was associated with reductions in mean body weight of at least 20% in male and female rats and 10% in male and female mice. Although a 13-week feeding study was also reported in the literature for 4-chloro-1,2-benzenediamine, limited details were available.

Chronic dietary administration of 4-chloro-1,2-benzenediamine resulted in increased incidences of urinary bladder carcinomas and papillomas in male and female rats and combined hepatocellular carcinomas and adenomas in male and female mice. Hepatic focal hyperplasia and renal pyelonephritis were among the non-neoplastic effects observed in rats at dose-related increased incidences compared to controls after chronic dietary administration of 4-chloro-1,2-benzenediamine. Uterine/endometrial hyperplasia was observed in female mice at an increased incidence compared to controls after chronic dietary administration of 4-chloro-1,2-benzenediamine. No reproduction or developmental toxicity studies were located.

The weight of evidence suggests that 4-chloro-1,2-benzenediamine is genotoxic *in vivo* and *in vitro*. 4-Chloro-1,2-benzenediamine was positive in the *Salmonella* reverse mutation assay with metabolic activation and was positive for mutation in hepatocytes isolated from the livers of Big Blue™ mice exposed *in vivo*. 4-Chloro-1,2-benzenediamine caused chromosomal aberrations *in vivo* in mouse bone marrow cells. It produced a few cells with very large numbers of aberrations, rather than an even distribution of damage among cells. 4-Chloro-1,2-benzenediamine produced dose-related increases in micronucleated cells *in vivo* in the mouse micronucleus assay, and also induced sister chromatid exchanges *in vivo*. 4-Chloro-1,2-benzenediamine produced a positive result in the *in vivo* alkaline single cell assay (Comet assay) in the liver, and was positive for unscheduled DNA synthesis in an *in vitro* DNA repair assay in

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primary rat hepatocytes. It was positive with metabolic activation in an *in vitro* microscreen prophage-induction assay in *Escherichia coli* $WP2_s(\lambda)$ for the ability to induce DNA damage.

8.1 Limited-Exposure Effects

No limited exposure studies assessing dermal or ocular irritation or dermal sensitization were identified for 4-chloro-1,2-benzenediamine.

8.2 Single-Exposure Studies

No LD_{50} or other acute toxicity studies were identified for 4-chloro-1,2-benzenediamine.

8.3 Short-Term Exposure Studies

According to the NTP (2004), Fischer 344 rats and B6C3F1 mice were administered 4-chloro-1,2-benzenediamine in the diet in a 14-day study, but no further information was available.

8.4 Long-Term and Chronic Exposure Studies

8.4.1 Subchronic Studies

Five Fischer 344 rats per sex and dose were administered 4-chloro-1,2-benzenediamine (purity unspecified) at dietary levels of 0, 0.03, 0.1, 0.3, 1.0, or 3.0% for eight weeks (NTP/NCI, 1978a). These doses were approximately equivalent to 0, 30, 100, 300, 1,000, or 3,000 mg/kg-day in males and 0, 34, 113, 340, 1,130, or 3,400 mg/kg-day in females. Individual body weights were recorded throughout the study. Food consumption was recorded during Weeks 1, 4, and 7. At study termination, all survivors were sacrificed and necropsied. At the 1% dietary level, mean body weight was depressed 29% in males and 20% in females. At the 3% dietary level, an unspecified number of rats died. This study was conducted to determine dose levels for a chronic feeding study and, as such, did not examine all the endpoints required under current regulatory guidelines (U.S. EPA, 2005b).

Five B6C3F1 mice per sex and dose were administered 4-chloro-1,2-benzenediamine (purity unspecified) at dietary levels of 0, 0.03, 0.1, 0.3, 1.0, or 3.0% for eight weeks (NTP/NCI, 1978a). These doses were approximately equivalent to 0, 54, 180, 540, 1,800, or 5,400 mg/kg-day in males and 0, 59, 195, 590, 1,693, or 5,900 mg/kg-day in females. Individual body weights were recorded throughout the study. Food consumption was recorded during Weeks 1, 4, and 7. At study termination, all survivors were sacrificed and necropsied. At the 1% dietary level, mean body weight was depressed 15% in males and 12% in females. At the 3% dietary level, an unspecified number of mice died. This study was conducted to determine dose levels for a chronic feeding study and, as such, did not examine all the endpoints required under current regulatory guidelines (U.S. EPA, 2005b).

Five Fischer 344 rats and five B6C3F1 mice per sex and dose were administered 4-chloro-1,2-benzenediamine (purity unspecified) at dietary levels of 0, 0.3, or 3.0% for 13-weeks. The study and study citation were not identified. Limited details were available in NTP (2004) and

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Weisburger et al. (1980). According to Weisburger et al. (1980), the highest dose causing no deaths, gross abnormalities, or reductions in body weight of > 30% was chosen as the highest dose for the chronic study by NTP/NCI (1978a).

8.4.2 Chronic Studies

8.4.2.1 Twenty-Six-Week Feeding Study in C57BL/6 Big Blue™ Mice

4-Chloro-1,2-benzenediamine (97.1% purity) was administered at dietary levels of 0, 5,000, or 10,000 ppm to groups of C57BL/6 Big Blue™ mice for 26 weeks (Suter et al., 1998). According to the authors, the resulting daily doses were 0, 1,794, or 3,926 mg/kg-day in males and 0, 2,539, or 5,925 mg/kg-day in females. These doses were equal to those from Week 34 onward from the cancer bioassay conducted by NTP/NCI (1978a). Food and water were available *ad libitum*. Mortality and clinical signs were monitored daily. Food consumption for a one-day period each week was measured. Body weights were recorded weekly. Gross necropsies were performed at study termination. Liver weights were measured and liver-to-body-weight ratios calculated. Results are presented in Table 2.

Table 2. Results of a 26-week feeding study in C57BL/6 Big Blue™ mice (Suter et al., 1998)

Effect	Response			
	5,000 ppm		10,000 ppm	
	Male	Female	Male	Female
Mean terminal body weight	↓ 29%	↓ 3%	↓ 22%	↓ 11%
Absolute liver weight	↓ 14%	↑ 9%	↑ 15%	↑ 19%
Relative liver weight	↑ 18% *	↑ 12% *	↑ 34% *	↑ 29% *
* Statistically significant result				

8.4.2.2 Two-Year Feeding Study in Fischer 344 Rats

In a study by the NTP/NCI, 50 six-week old Fischer 344 rats per sex and dose were administered 4-chloro-1,2-benzenediamine at dietary levels of 0, 0.5, or 1% (approximately equivalent to 0, 400, or 833 mg/kg-day in males and 0, 350, or 724 mg/kg-day in females; Section 13.1) for 78 weeks (NTP/NCI, 1978a; Weisburger et al., 1980; Sontag, 1981). The compound was qualitatively determined to be "of fairly high purity" by several methods, with up to two unidentified impurities detected. The doses were selected based on an eight-week feeding study, which was conducted to approximate the maximum tolerated dose. After the 78-week period of compound administration, observation of the rats continued for an additional 28 weeks. Rats were housed five per cage by sex. Food and water were available *ad libitum*. Mortality was monitored daily. Body weights were measured twice weekly for the first 12 weeks and monthly thereafter. Food consumption of two cages per dose was measured for seven consecutive days once a month for the first nine months, and for three consecutive days once a month each month thereafter. Five rats per sex from the control and high-dose groups were sacrificed after 78 weeks, and the remaining rats were sacrificed at study termination (106 weeks). Gross necropsies were performed on all rats that died or were sacrificed moribund during the study, and

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on all remaining rats at study termination. Histopathological examination included the adrenals, bone marrow, brain, bronchi, cecum, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, mammary glands, ovaries, pancreas, pituitary, prostate, salivary gland, seminal vesicle, subcutaneous tissue, skin, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, Zymbal gland, and gross lesions. The authors noted that a few organs (unspecified) were not histopathologically examined in some rats, particularly those that died prior to study termination, or those that were missing, cannibalized, and/or judged to be in an advanced state of autolysis.

This study was conducted prior to the development of current regulatory guidelines, and some deficiencies exist (U.S. EPA, 2005b), since dosing did not continue for 24 months. Further, although five rats per sex from the control and high-dose groups were sacrificed after 78 weeks, presumably for necropsy and histology, the tabulated results for these rats were included with the results from the remaining rats, which were sacrificed at the 106-week study termination. Body weight and food consumption were not measured weekly, and hematology, clinical chemistry, and urinalysis were not conducted. Organ weights were not measured, and histology did not include the aorta, bone, eye, muscle, nerve, or spinal cord.

Neoplastic and non-neoplastic effects observed in treated but not control rats, or observed at a higher incidence in treated rats compared to control rats, are summarized in Table 3.

Since the individual and combined tumor incidences were statistically increased at the mid- and high doses in male and female rats, the authors concluded that chronic dietary administration of 4-chloro-1,2-benzenediamine resulted in increased incidences of urinary bladder carcinomas and papillomas in male and female rats. However, it should also be noted that at the time this cancer study was conducted, the procedure was to house the test animals in a room with animals dosed with other chemicals in other studies. The study authors did not note whether rats from other studies were housed in the same room as the rats from this study. Therefore, any effect of such combined housing, if it occurred, cannot be determined.

8.4.2.3 Two-Year Feeding Study in $B6C3F_1$ Mice

Fifty $B6C3F_1$ mice per sex and dose were administered 4-chloro-1,2-benzenediamine at dietary levels of 0, 1, or 2% for 33 weeks and then at 0, 0.5, or 1% through the 78th week (NTP/NCI, 1978a; Weisburger et al., 1980; Sontag, 1981). The time-weighted average dietary concentrations for low and high dose mice were 0, 0.7, or 1.4%, respectively. These percentages were approximately equivalent to 0, 1,180, or 2,635 mg/kg-day in males and 0, 1,377, or 2,847 mg/kg-day in females (Section 13.1). The compound was qualitatively determined to be "of fairly high purity" by several methods, with up to two unidentified impurities detected. The doses were selected based on an eight-week feeding study, which was conducted to approximate the maximum tolerated dose. After the 78-week period of compound administration, the mice were observed for an additional 17-18 weeks. Mice were housed 10 per cage by sex for the first 11 months and five per cage thereafter. Food and water were available *ad libitum*. Mortality was monitored daily. Body weights were measured twice weekly for the first 12 weeks and monthly thereafter. Food consumption of two cages per dose was measured for seven consecutive days once a month for the first nine months, and for three consecutive days once a

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