United States
Environmental Protection
Agency

FINAL DRAFT
ECAO-CIN-G087
JULY, 1990

Research and Development

HEALTH AND ENVIRONMENTAL EFFECTS DOCUMENT FOR 4,4'-METHYLENE-BIS(2-CHLOROANILINE)

Prepared for

OFFICE OF SOLID WASTE AND
EMERGENCY RESPONSE

Prepared by

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PREFACE

Health and Environmental Effects Documents (HEEDs) are prepared for the Office of Solid Waste and Emergency Response (OSWER). This document series is intended to support listings under the Resource Conservation and Recovery Act (RCRA) as well as to provide health-related limits and goals for emergency and remedial actions under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained for Agency Program Office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched for in this document and the dates searched are included in "Appendix: Literature Searched." Literature search material is current up to 8 months previous to the final draft date listed on the front cover. Final draft document dates (front cover) reflect the date the document is sent to the Program Officer (OSWER).

Several quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic and subchronic exposures for both the inhalation and oral exposures. The subchronic or partial lifetime RfD is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval i.e., for an interval that does not constitute a significant portion of the lifespan. This type of exposure estimate has not been extensively used, or rigorously defined as previous risk assessment efforts have focused primarily on lifetime exposure scenarios. Animal data used for subchronic estimates generally reflect exposure durations of 30-90 days. The general methodology for estimating subchronic RfDs is the same as traditionally employed for chronic estimates, except that subchronic data are utilized when available.

In the case of suspected carcinogens, a carcinogenic potency factor, or q_1^* (U.S. EPA, 1980), is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively. An RfD may also be derived for the noncarcinogenic health effects of compounds that are also carcinogenic.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity, and acute mammalian toxicity). Chemical-specific RQs reflect the lowest of these six primary criteria. The methodology for chronic toxicity and cancer based RQs are defined in U.S. EPA, 1984 and 1986a, respectively.

EXECUTIVE SUMMARY

4,4'-Methylene-bis(2-chloroaniline) is a solid at room temperature. It is slightly soluble in water and is soluble in most common organic solvents (Sax and Lewis, 1987; Windholz et al., 1983). According to U.S. EPA TSCA production file, three companies produced or imported between 2.01 and 20.1 million pounds of 4,4'-methylene-bis(2-chloroaniline) in 1977 (TSCAPP, 1989). U.S. production of this compound ceased by 1980. Since 1980, all 4,4'-methylene-bis(2-chloroaniline) used in the United States is imported from Japan (Ward et al., 1987). 4,4'-Methylene-bis(2-chloroaniline) is used as a curing agent for both liquid-castable polyurethane elastomers and epoxy resins (Fishbein, 1984; Sax and Lewis, 1987).

4,4'-Methylene-bis(2-chloroaniline) is expected to exist predominantly in the particulate form in the ambient atmosphere. Pertinent data regarding the atmospheric fate of 4,4'-methylene-bis(2-chloroaniline) were limited in the available literature. Deposition of both particulate and adsorbed 4,4'-methylene-bis(2-chloroaniline) is expected to be the dominant fate process in the atmosphere. The gas-phase reaction of 4,4'-methylene-bis-(2-chloroaniline) with ozone is not expected to be significant and the gas phase reaction with photochemically produced HO• is expected to be rapid for the small proportion of this compound existing in the vapor phase. If released to water, 4,4'-methylene-bis(2-chloroaniline) is expected to adsorb strongly to sediment and suspended organic matter. It may moderately bioaccumulate in fish and aquatic organisms. Neither hydrolysis nor volatilization to the atmosphere is expected to be significant. Conflicting data on the biodegradation of 4,4'-methylene-bis(2-chloroaniline) under aerobic conditions were found; therefore, its fate by this process is

unknown. 4,4'-Methylene-bis(2-chloroaniline) is expected to adsorb strongly to soil. It may form covalent bonds with the active sites of the soil. A single study suggests that aerobic biodegradation in soil may occur after a short induction period. 4,4'-Methylene-bis(2-chloroaniline) is not expected to volatilize from the soil surface to the atmosphere.

Limited data are available regarding exposure to 4,4'-methylene-bis-(2-chloroaniline). It appears that occupational exposure by dermal contact will predominate; however, inhalation of particulate 4,4'-methylene-bis-(2-chloroaniline) is also possible. High levels of this compound have been found on indoor surfaces where it is stored or used commercially. The lack of ambient monitoring data on levels of 4,4'-methylene-bis(2-chloroanaline) does not allow the determination of the level of human exposure. The monitoring data located in the literature are concerned with the levels of 4,4'-methylene-bis(2-chloroaniline) near a major manufacturing site no longer producing this compound. Thus, populations that reside near facilities that use 4,4'-methylene-bis(2-chloroaniline) may be exposed to the compound. Sufficient data could not be located to accurately predict levels of exposure to the general population.

No data were located regarding the environmental toxicity of 4,4'-methylene-bis(2-chloroaniline).

Excretion data on rats indicate that 4,4'-methylene-bis(2-chloroaniline) is rapidly absorbed from the gastrointestinal tract (Farmer et al., 1981). It has been shown that 4,4'-methylene-bis(2-chloroaniline) can be absorbed through the skin of rabbits (E.I. DuPont de Nemours and Company, Inc., 1977), dogs (Manis et al., 1984) and humans (Osorio et al., 1986). In the dog study, absorption was estimated at 2.4-10% of the applied dose over a 24-hour exposure period. Absorbed 4,4'-methylene-bis(2-chloroaniline) is

rapidly distributed throughout the body. Highest levels, regardless of route of administration, are located in the liver and fat, but no organ or tissue appears to preferentially accumulate or retain 4,4'-methylene-bis-(2-chloroaniline) or its metabolites.

Farmer et al. (1981) determined that both low and high doses of 4,4'-methylene-bis(2-chloroaniline) were extensively metabolized by the same metabolic systems in rats. They showed that the major urinary products were conjugates of several metabolites and that only 1-2% was excreted unchanged in the urine. In the urine of humans with known exposure to 4,4'-methylene-bis(2-chloroaniline), however, only unmetabolized compound was identified in the urine and the investigators concluded that important species differences exist in the metabolism of the compound. In vitro studies, however, indicate that liver microsomal preparations from rats and humans biotransform 4,4'-methylene-bis(2-chloroaniline) to the N-hydroxy, 6-hydroxy and benzhydrol derivatives (Morton et al., 1986).

Based on accidental human dermal exposure to 4,4'-methylene-bis-(2-chloroaniline), the half-life in a human was estimated as ~23 hours (Osorio et at., 1986). Studies using rats (Farmer et al., 1981; Tobes et al., 1983) indicate that excretion is rapid after intravenous, intraperitoneal or oral administration. Fecal excretion exceeds urinary excretion roughly by a factor of two regardless of route of administration. A distribution study reported that 32% of the dose given to dogs was located in the bile 24 hours after intravenous treatment, indicating that biliary excretion is important in the elimination of 4,4'-methyelene-bis(2-chloroaniline).

The carcinogenic effects of 4,4'-methylene-bis(2-chloroaniline) administered in the diets of mice, rats and dogs have been the subject of several studies. Results have shown that administration in the diet to CD-1 mice

produced increased incidences of hepatomas in females compared with controls (Russfield et al., 1975) (see Table 6-1). When given in the diet to male and female Charles River CD rats, 4,4'-methylene-bis(2-chloroaniline) produced statistically significant increased incidences of lung adenocarcinomas compared with controls (Stula et al., 1975) (see Table 6-2). Kommineni et al. (1979) reported that dietary 4,4'-methylene-bis(2-chloroaniline) given to male Charles River Sprague-Dawley rats for 18 months followed by 6 months of observation led to increased incidences of pulmonary adenomas and adenocarcinomas, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas compared with controls (see Table 6-3). Four of five female beagle dogs given 4,4'-methylene-bis(2-chloro- aniline) in gelatin capsules for $\leq$9 years developed urinary bladder tumors while no control dogs developed such tumors (Stula et al., 1977) (see Table 6-4).

4,4'-Methylene-bis(2-chloroaniline) has been shown to be mutagenic or genotoxic in bacterial and yeast assays, with or without microsomal activation (Takemura and Shimizu, 1978; Ho et al., 1979; Shimizu et al., 1982; McCann et al., 1975; Ichinotsubo et al., 1981; Ho et al., 1979). It failed to induce sex-linked recessive lethal mutations in Drosophila melanogaster (Ho et al., 1979), and it did not produce chromatid aberrations or SCE in Chinese hamster ovary cells or human leucocytes (Galloway et al., 1985; Ho et al., 1979) (see Table 6-5). It was reported to produce unscheduled DNA synthesis in rodent and rabbit hepatocytes (McQueen et al., 1981, 1983; McQueen and Williams, 1982; Mori et al., 1988).

Occupational exposure to 4,4'-methylene-bis(2-chloroaniline) has been associated with a reversible form of hematuria, but exposures were not precisely quantified (Mastromatteo, 1965). Signs of systemic toxicity from oral administration of 4,4'-methylene-bis(2-chloroaniline) in animals

included the following: liver injury in female beagle dogs administered 7.3 mg/kg/day for 9 years (Stula et al., 1977); high mortality in female CD-1 mice at dietary concentrations of 2000 ppm for 18 months followed by 6 months on a normal diet (Russfield et al., 1975); decreased body weight gains in CD-1 male rats administered 500 or 1000 ppm for 18 months followed by 6 months of observation (Russfield et al., 1975); decreased survival time in CR Sprague-Dawley rats administered 4,4'-methylene-bis(2-chloroaniline) in the diet at 0, 250, 500 and 1000 ppm for 18 months and observed for 6 months (Kommineni et al., 1979) and liver effects including hepatocytomegaly, fatty change, necrosis, bile duct proliferation and fibrosis in Charles River CD rats administered 1000 ppm in the diets for $\leq$2 years (Stula et al., 1975).

In vitro studies with rat liver cells (Silk et al., 1989), and dog and human bladder explant cultures (Stoner et al., 1987) have led to the isolation of 4,4'-methylene-bis(2-chloroaniline)-DNA adducts, suggesting that 4,4'-methylene-bis(2-chloroaniline) can bind to DNA in these species. Three of the adducts were common to dog and human cell cultures.

Because of positive evidence regarding the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) when administered orally to rats, mice, and dogs, and when administered subcutaneously to rats, the compound was assigned to U.S. EPA Group B2: probable human carcinogen. A human q_1^* of 1.3×10^{-1} $(mg/kg/day)^{-1}$ was derived for oral exposure to 4,4'-methylene-bis(2-chloroaniline) and was also adopted for inhalation exposure. An air concentration of 5.4×10^{-4} mg/m^3 would be associated with increased cancer risk of 1×10^{-5} and a concentration of 2.7×10^{-3} mg/ℓ in drinking water would be associated with increased cancer risk of 1×10^{-5}. An RQ for carcinogenicity of 100 was based on the incidence of lung tumors in rats in studies by Stula et al. (1975) and Kommineni et al. (1979).

Pertinent data regarding the developmental and reproductive toxicity of 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature.

An RfD for subchronic and chronic oral exposures of 0.0007 mg/kg/day was based on the LOAEL that resulted in liver injury and bladder inflammation in dogs treated for 9 years at 7.3 mg/kg/day (Stula et al., 1977). An RQ of 1000 for chronic (noncancer) toxicity was based on increased mortality in male rats given 4,4'-methylene-bis(2-chloroaniline) in the diet for 18 months (Kommineni et al., 1979).

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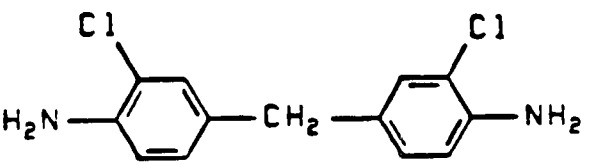
LIST OF ABBREVIATIONS

AEL	Adverse-effect level
BCF	Bioconcentration factor
bw	Body weight
CAS	Chemical Abstract Service
CS	Composite score
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
FEL	Frank effect level
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
K_{oc}	Soil sorption coefficient standardized with respect to organic carbon
K_{ow}	Octanol/water partition coefficient
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactate dehydrogenase
LED	Lowest effective dose
LOAEL	Lowest-observed-adverse-effect level
NOAEL	No-observed-adverse-effect level
ppb	Parts per billion
ppm	Parts per million
MTD	Maximum tolerated dose
RfD	Reference dose
SCE	Sister chromatid exchange
TLV	Threshold limit value
TWA	Time weighted average
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

4,4'-Methylene-bis(2-chloroaniline) is known by the synonyms bis amine bis(3-chloro-4-aminophenyl)methane, bis(4-amino-3-chlorophenyl)methane and methylene-bis-ortho-chloroaniline; the trade names Cuamine MT, Curalin M, Curene 442 and Cyanaset; and the acronyms CL-MDA and DACPM (Chemline, 1989). The structure, CAS number, empirical formula and molecular weight are given below:



CAS Registry number: 101-14-4

Empirical formula: $C_{13}H_{12}Cl_2N_2$

Molecular weight: 267.16

1.2. PHYSICAL AND CHEMICAL PROPERTIES

4,4'-Methylene-bis(2-chloroaniline) is a solid at room temperature. It is slightly soluble in water and is soluble in dilute acids, ether, alcohol, methylethylketone, acetone, esters and aromatic hydrocarbons (Sax and Lewis, 1987; Windholz et al., 1983). Selected physical and chemical properties of 4,4-methylene-bis(2-chloroaniline) are as follows:

Melting point:	110°C	Windholz et al., 1983
Water solubility at 24°C:	139 mg/ℓ	Voorman and Penner, 1986
Log K_{ow}:	3.94	U.S. EPA, 1987a
Vapor pressure at 25°C:	1.32×10^{-8} mm Hg	U.S. EPA, 1987b
Conversion factors at 25°C:	1 mg/m³ = 9.16×10^{-2} ppm; 1 ppm = 10.9 mg/m³	

1.3. PRODUCTION DATA

This compound was produced or imported in 1977 by Polyester Corporation of Southampton, NY; Andersen Development Co., of Adrian, MI; and E.I. Dupont and De Nemours and Co., of Deepwater, NJ. The total production volume was between 2.01 and 20.1 million pounds (one plant's production volume was listed as confidential) (TSCAAP, 1989). These companies ceased production by 1980. Since 1980, all 4,4'-methylene-bis(2-chloroaniline) used in the United States has been imported from Japan (Ward et al., 1987). It has been estimated that 200-400 U.S. firms are engaged in the production of products cured with 4,4'-methylene-bis(2-chloroaniline) (Ward et al., 1987).

4,4'-Methylene-bis(2-chloroaniline) was produced commercially in the United States by the condensation of formaldehyde with two equivalents of ortho-chloroaniline (Fishbein, 1984).

1.4. USE DATA

4,4'-Methylene-bis(2-chloroaniline) is perhaps the most widely used curing agent for both epoxy resins and liquid-castable polyurethane elastomers suitable for molded mechanical articles and for potting and encapsulating purposes (Fishbein, 1984; Sax and Lewis, 1987).

1.5. SUMMARY

4,4'-Methylene-bis(2-chloroaniline) is a solid at room temperature. It is slightly soluble in water and is soluble in most common organic solvents (Sax and Lewis, 1987; Windholz et al., 1983). According to U.S. EPA TSCA production file, three companies produced or imported between 2.01 and 20.1 million pounds of 4,4'-methylene-bis(2-chloroaniline) in 1977 (TSCAPP, 1989). U.S. production of this compound ceased by 1980. Since 1980, all 4,4'-methylene-bis(2-chloroaniline) used in the United States is imported

from Japan (Ward et al., 1987). 4,4'-Methylene-bis(2-chloroaniline) is used as a curing agent for both liquid-castable polyurethane elastomers and epoxy resins (Fishbein, 1984; Sax and Lewis, 1987).

2. ENVIRONMENTAL FATE AND TRANSPORT

2.1. AIR

The estimated vapor pressure of 4,4'-methylene-bis(2-chloroaniline), 1.32×10^{-8} mm Hg at 25°C (U.S. EPA, 1987b), suggests that this compound will exist partially in the vapor phase, but predominantly in the particulate form in the ambient atmosphere (Eisenreich et al., 1981).

2.1.1. Reaction with HO•. Using a method of Atkinson (1985), the rate for the gas phase reaction of 4,4'-methylene-bis(2-chloroaniline) with photochemically produced HO• can be estimated at 1.22×10^{-10} cm^3/molecule-second. If the average atmospheric HO• concentration is $1 \times 10^{+5}$ molecules/cm^3, then a half-life of 0.132 days can be calculated (Atkinson, 1985). However, 4,4'-methylene-bis(2-chloroaniline) is expected to exist predominately in the particulate form in the ambient atmosphere and only small amounts will exist in the vapor phase. Particulate 4,4'-methylene-bis(2-chloroaniline) is expected to be less chemically reactive. The actual rate of destruction of total 4,4'-methylene-bis(2-chloroaniline) by photochemically produced HO• will therefore be considerably slower.

2.1.2. Reaction with Ozone. Quantitative data regarding the gas-phase reaction of 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A. In water, 4,4-methylene-bis-(2-chloroaniline) in water has been reported to undergo $\leq$1% reaction with ozone after 130 minutes (Fochtman and Eisenberg, 1979). Therefore, the gas phase destruction of 4,4'-methylene-bis(2-chloroaniline) by the reaction with ozone is not expected to be a significant process.

2.1.3. Photolysis. Pertinent data regarding the direct destruction of 4,4'-methylene-bis(2-chloroaniline) by photolysis were not located in the available literature cited in Appendix A. Generally, aromatic amines adsorb

UV light at wavelengths >290 nm, which is the UV region of the electromagnetic spectrum found in the atmosphere. Therefore, 4,4'-methylene-bis-(2-chloroaniline) may be a candidate for direct photochemical degradation in the atmosphere.

2.1.4. Physical Removal Processes. Quantitative data regarding the physical removal of 4,4'-methylene-bis(2-chloroaniline) from the atmosphere were not located in the available literature cited in Appendix A. The water solubility of 4,4'-methylene-bis(2-chloroaniline), 13.9 mg/ℓ at 24°C (Voorman and Penner, 1986), suggests that rain washout may occur. Dry deposition of adsorbed particulate 4,4'-methylene-bis(2-chloroaniline) is also expected to be a significant removal process.

2.2. WATER

2.2.1. Hydrolysis. An experimental rate constant for the hydrolysis of 4,4'-methylene-bis(2-chloroaniline) at neutral pH has been determined as $<9x10^{-8}$ 1/hour, which is a half-life of >800 years. Under acidic conditions, an experimental rate of $2.9x10^{-4}$ ℓ/mole-hour equals a half-life of 4000 years at pH 5 (Ellington et al., 1988). Thus, hydrolysis of 4,4'-methylene-bis(2-chloroaniline) is not expected to be a significant fate process.

2.2.2. Oxidation. Pertinent data regarding the chemical oxidation of 4,4'-methylenebis(2-chloroaniline) in water were not located in the available literature cited in Appendix A.

2.2.3. Photolysis. Pertinent data regarding the photolytic destruction of 4,4'-methylene-bis(2-chloroaniline) in water were not located in the available literature cited in Appendix A.

2.2.4. Microbial Degradation. In a screening test using settled domestic wastewater sludge under aerobic conditions, the author reported that

4,4'-methylene-bis(2-chloroaniline) was not biologically dissimilated effectively after the sixth subculture and enrichment process (Tabak et al., 1980). In another static test using a settled domestic wastewater inoculum, 2.0 mg/ℓ of 4,4'-methylene-bis(2-chloroaniline) was not biologically decomposed effectively under aerobic conditions. When the authors utilized a continuous biological reactor with the same inocula, 4,4'-methylene-bis-(2-chloroaniline) was reduced from 2.02 to 0.09 mg/ℓ in 24 hours, which the authors reported as loss from biodegradation (Fochtman and Eisenberg, 1979). However, no suitable control experiment was performed and it is possible that the loss of 4,4'-methylene-bis(2-chloroaniline) was due to adsorption to the sludge rather than to biodegradation. Others have reported that 4,4'-methylene-bis(2-chloroaniline) can concentrate in the sludge of waste water treatment plants (Parris et al., 1980).

Pure cultures of Norcardiopsis sp. and Bacillus megaterium degraded 4,4'-methylene-bis(2-chloroaniline) to products resulting from N-acylation or N-hydroxylation of the aromatic nitrogen (Yoneyama and Matsumura, 1984).

2.2.5. Bioconcentration. The BCF of an organic compound is directly related to its K_{ow}. Using the regression equation log BCF = 0.76 log K_{ow} - 023 (Bysshe, 1982), a value of 581 can be calculated for 4,4'-methylene-bis(2-chloroaniline) using an estimated K_{ow} of 3.94 (U.S. EPA, 1987a). This value suggests that 4,4'-methylene-bis(2-chloroaniline) is expected to moderately bioaccumulate in fish and aquatic organisms.

2.2.6. Adsorption. Since 4,4'-methylene-bis(2-chloroaniline) has a strong affinity for soil (Section 2.3.2.), this compound is expected to significantly adsorb to sediment and suspended organic matter.

2.2.7. Volatilization. Using the bond contribution method of Hine and Mookerjee (1975), a Henry's Law constant of 4.06×10^{-11} atm-m³/mole can

be estimated for 4,4'-methylene-bis(2-chloroaniline). This value suggests that volatilization from water to the atmosphere will not be an important process (Thomas, 1982); the volatilization rate will be so slow that 4,4'-methylene-bis(2-chloroaniline) will be expected to concentrate in solution as the water evaporates.

2.3. SOIL

2.3.1. Microbial Degradation. 4,4'-Methylene-bis(2-chloroaniline) underwent microbial degradation to bis(2-chloroaniline)methone, the corresponding benzophenone analog, in Hoytville soil under aerobic conditions after a 3-day induction period. No rate information was presented in this study and complete mineralization of 4,4'-methylene-bis(2-chloroaniline) to carbon dioxide did not occur (Voorman and Penner, 1986).

2.3.2. Adsorption. 4,4'-Methylene-bis(2-chloroaniline) rapidly binds to the soil matrix and probably forms covalent adducts with the active sites of soil particles (Voorman and Penner, 1986). Generally, aromatic amines are known to covalently bond to soil (Parris, 1980). When ^{14}C-labeled 4,4'-methylene-bis(2-chloroaniline) was applied to Hoytville soil at a concentration of 4 mg/kg, only 30% of the original radioactivity was extracted after 24 hours (extraction efficiency at time zero: 85-95%). At an application rate of 40 mg/kg, ~45% of the applied material was recovered after 24 hours. The loss was not a result of biodegradation since the induction period in these experiments was 3 days (see Section 2.3.1.) (Voorman and Penner, 1986). From the Freundlich constant (Kp=354) presented in this paper, a K_{oc} of 4600 can be calculated (Lyman, 1982). This K_{oc} value suggests that 4,4'-methylene-bis(2-chloroaniline) will display slight mobility in soil (Swann et al., 1983).

2.3.3. Volatilization. The vapor pressure of 4,4'-methylene-bis-(2-chloroaniline), 1.32×10^{-8} mm Hg at 25°C (U.S. EPA, 1987b), in combination with its strong adsorption to soil (Voorman and Penner, 1986), suggests that volatilization from the soil surface to the atmosphere will not be significant.

2.4. SUMMARY

4,4'-Methylene-bis(2-chloroaniline) is expected to exist predominantly in the particulate form in the ambient atmosphere. Pertinent data regarding the atmospheric fate of 4,4'-methylene-bis(2-chloroaniline) were limited in the available literature. Deposition of both particulate and adsorbed 4,4'-methylene-bis(2-chloroaniline) is expected to be the dominant fate process in the atmosphere. The gas-phase reaction of 4,4'-methylene-bis-(2-chloroaniline) with ozone is not expected to be significant and the gas phase reaction with photochemically produced HO• is expected to be rapid for the small proportion of this compound existing in the vapor phase. If released to water, 4,4'-methylene-bis(2-chloroaniline) is expected to adsorb strongly to sediment and suspended organic matter. It may moderately bioaccumulate in fish and aquatic organisms. Neither hydrolysis nor volatilization to the atmosphere is expected to be significant. Conflicting data on the biodegradation of 4,4'-methylene-bis(2-chloroaniline) under aerobic conditions were found; therefore, its fate by this process is considered uncertain. In soil, 4,4'-methylene-bis(2-chloroaniline) is expected to adsorb strongly to soil. It may form covalent bonds with the active sites of soil particles. A single study suggests that aerobic biodegradation in soil may occur after a short induction period. 4,4'-Methylene-bis(2-chloroaniline) is not expected to volatilize from the soil surface to the atmosphere.

3. EXPOSURE

3.1. WATER

At the site of a major producer of this compound near Adrian, MI, 4,4'-methylene-bis(2-chloroaniline) was found in the industrial lagoon sediment at a minimum concentration of 1600 ppm (dry weight). It was detected in the lagoon effluent water at a concentration of 250 ppb, and in deep well water on the manufacturing site at a concentration of 1.5 ppb. Surface runoff water at the plant contained 1 ppb of 4,4'-methylene-bis-(2-chloroaniline). At the Adrian, MI, sewage treatment plant, the influent and effluent water had a detectable, but not quantifiable, amount of 4,4'-methylene-bis(2-chloroaniline). The activated sludge from this treatment plant had an estimated concentration of 18 ppm (dry weight) (Parris et al., 1980).

3.2. FOOD

Pertinent data regarding 4,4'-methylene-bis(2-chloroaniline) in fish were not located in the available literature cited in Appendix A.

3.3. INHALATION

The lack of air monitoring data precludes the determination of worker exposure to 4,4'-methylene-bis(2-chloroaniline) (Ward et al., 1987). Occupational exposure by inhalation of airborne dust can occur during the transfer of 4,4'-methylene-bis(2-chloroaniline) from the container in which it was shipped (Schulte et al., 1988).

3.4. DERMAL

Although monitoring data are lacking, the most serious route of exposure to 4,4'-methylene-bis(2-chloroaniline) is believed to be through the skin (Schulte et al., 1988). In a study attempting to correlate 4,4'-methylene-bis(2-chloroaniline) air levels with its concentration in the urine of

workers at a plant where it was used commercially, only 15% of the samples were above the limits of analytical detection, 0.01 mg/m³. The concentration in the urine of workers, however, ranged from 70-1500 µg/ℓ. This was interpreted as a result of absorption through the skin (Schulte et al., 1988; Ward et al., 1987). Wipe samples of the work surfaces at facilities using 4,4'-methylene-bis(2-chloroaniline) showed surface concentrations ≤15,000 µg/100 cm² in areas where it was stored or used (Ward et al., 1987).

3.5. OTHER

The area surrounding an Adrian, MI, production facility of 4,4'-methylene-bis(2-chloroaniline) had soil levels of this compound ranging from 1.6-200 ppm as monitored from a variety of samples (Fishbein, 1984).

3.6. SUMMARY

Limited data are available regarding exposure to 4,4'-methylene-bis-(2-chloroaniline). It appears that occupational exposure by dermal contact will predominate; however, inhalation of particulate 4,4'-methylene-bis-(2-chloroaniline) is also possible. High levels of this compound have been found on indoor surfaces where it is stored or used commercially. The lack of ambient monitoring data on levels of 4,4'-methylene-bis(2-chloroanaline) does not allow the determination of the level of human exposure. The monitoring data located in the literature are concerned with the levels of 4,4'-methylene-bis(2-chloroaniline) near a major manufacturing site no longer producing this compound. Thus, populations that reside near facilities that use 4,4'-methylene-bis(2-chloroaniline) may be exposed to the compound. Sufficient data could not be located to accurately predict levels of exposure to the general population.

4. ENVIRONMENTAL TOXICOLOGY

4.1. AQUATIC TOXICOLOGY

4.1.1. Acute Toxic Effects on Fauna. Pertinent data regarding the effects of acute exposure of aquatic fauna to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.1.2. Chronic Effects on Fauna.

4.1.2.1. TOXICITY -- Pertinent data regarding the effects of chronic exposure of aquatic fauna to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.1.2.2. BIOACCUMULATION/BIOCONCENTRATION -- Pertinent data regarding the bioaccumulation/bioconcentration potential of 4,4'-methylene-bis-(2-chloroaniline) in aquatic fauna were not located in the available literature cited in Appendix A.

4.1.3. Effects on Flora.

4.1.3.1. TOXICITY -- Pertinent data regarding the toxic effects of exposure of aquatic flora to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.1.3.2. BIOCONCENTRATION -- Pertinent data regarding the bioconcentration potential of 4,4'-methylene-bis(2-chloroaniline) in aquatic flora were not located in the available literature cited in Appendix A.

4.1.4. Effects on Bacteria. Pertinent data regarding the effects of exposure of aquatic bacteria to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.2. TERRESTRIAL TOXICOLOGY

4.2.1. Effects on Fauna. Pertinent data regarding the effects of exposure of terrestrial fauna to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.2.2. Effects on Flora. Pertinent data regarding the effects of exposure of terrestrial flora to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

4.3. FIELD STUDIES

Pertinent data regarding the effects of 4,4'-methylene-bis(2-chloroaniline) on flora and fauna in the field were not located in the available literature cited in Appendix A.

4.4. AQUATIC RISK ASSESSMENT

No data were located regarding the effects of exposure of freshwater fauna and flora to 4,4'-methylene-bis(2-chloroaniline). Acute studies with representatives from eight families of freshwater fauna and at least three chronic studies and one bioconcentration study with freshwater fauna and flora are needed to develop a freshwater criterion by the method of U.S. EPA/OWRS (1986).

Pertinent data regarding the effects of exposure of marine fauna and flora to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A. Acute studies with representatives from eight families of marine fauna and at least three chronic studies and one bioconcentration study with marine fauna and flora are needed to develop a saltwater criterion by the method of U.S. EPA/OWRS (1986).

4.5. SUMMARY

No data were located regarding the environmental toxicity of 4,4'-methylene-bis(2-chloroaniline).

5. PHARMACOKINETICS

5.1. ABSORPTION

Farmer et al. (1981) administered to female LAC:Porton rats a single 10 mg/kg gavage dose of methylene-labeled [^{14}C]4,4'-methylene-bis(2-chloroaniline) in arachis oil. Urine and feces were collected for 48 hours. Urinary excretion accounted for 23.8% and fecal excretion for 59.5% of the dose of radioactivity. Urinary excretion accounted for 21.1% of the dose at 24 hours. Radioactivity in several tissues accounted for ~2.5% of the dose. These investigators also reported that fecal excretion accounted for 69.1% of the dose of radioactivity from a 1 mg/kg intraperitoneal injection. They concluded that gastrointestinal absorption was rapid.

Chin et al. (1983) studied the percutaneous absorption of [^{14}C]methylene-labeled 4,4'-methylene-bis(2-chloroaniline) by exposing organ cultures of human neonatal foreskin to [^{14}C]4,4'-methylene-bis(2-chloroaniline) bearing surfaces for $\leq$4 hours and monitoring the penetration of radioactivity into the cultures as a function of time and temperature. Foreskin integrity was insured by histological examination. Results showed that [^{14}C]4,4'-methylene-bis(2-chloroaniline) was absorbed quickly and progressively through the skin and that the rate of absorption was temperature dependent. Thin-layer chromatography confirmed that 4,4'-methylene-bis(2-chloroaniline) was not metabolized by the foreskin organ cultures. Accidental exposure data discussed in Section 5.4. (Osorio et al., 1986) provide qualitative evidence for percutaneous absorption in humans.

Following cutaneous application of ~10.5 mg [^{14}C]methylene-labeled 4,4'-methylene-bis(2-chloroaniline) in 0.5 mL acetone to a 25 cm^2 area of shaved dog skin, radioactivity in whole blood or plasma was not detected in the 24-hour monitoring period (Manis et al., 1984). At the end of 24

hours, urine contained 1.3% of the dose of radioactivity, bile contained 0.62% and skin at the application site contained 90%. By comparing excretion following cutaneous and intravenous administration, the authors estimated that 2.4-10% of the administered dose was absorbed into the systemic circulation in 24 hours.

A study with rabbits showed that 4,4'-methylene-bis(2-chloroaniline) can be absorbed dermally in this species as well (E.I. DuPont de Nemours and Company, Inc., 1977). No other details of this study are available.

5.2. DISTRIBUTION

A study measuring tissue distribution of [^{14}C]4,4'-methylene-bis-(2-chloroaniline) after a single intravenous administration to rats was conducted by Tobes et al. (1983). Twenty-one rats were lightly anesthetized with ether and injected with 0.49 mg/kg [^{14}C]4,4'-methylene-bis(2-chloroaniline). After 10 minutes, 1-, 4- and 16-hour blood samples were taken, and 5-6 rats/time period were anaesthetized with ether and sacrificed; samples from selected tissues were excised, weighed and analyzed for total radioactivity. Results are shown in Table 5-1. Because radioactivity was seen in several tissues 10 minutes after administration, the authors concluded that distribution occurred very quickly. Levels in adiposa and skin higher at 1 hour than at 10 minutes suggested a shift in distribution to organs of higher lipid content. Higher levels in the small intestine suggested enteric absorption of biliary excretion products. Analysis of radioactivity levels in fractions of rat liver cells 1 hour after intravenous administration indicated that the activity was evenly distributed within the cells.

Farmer et al. (1981) conducted a study to investigate the distribution of [^{14}C]4,4'-methylene-bis(2-chloroaniline) in female LAC:Porton rats

TABLE 5-1

Time-Dependent Tissue Distribution of Radioactivity in Rats After Intravenous Administration of 0.49 mg/kg [^{14}C]4,4'-Methylene-bis-(2-Chloroaniline)

Tissue	Mean Tissue Concentration, % kg dose/g[b] Time After Administration			
	10 minutes	1.0 hour	4.0 hours	16.0 hours
Adipose	0.064	0.183	0.145	0.041
Adrenals	0.385	0.114	0.007	0.041
Bladder	0.066	0.020	0.027	0.005
Brain				
grey	NR	0.028	NR	NR
white	NR	0.049	NR	NR
Intestine				
large	0.070	0.059	0.012	0.010
small	0.225	0.264	0.300	0.016
Kidney	0.185	0.135	0.092	0.061
Liver	0.496	0.246	0.169	0.107
Lung	0.249	0.170	0.126	0.074
Muscle	NR	0.032	NR	
Ovaries	0.194	0.049	0.040	0.026
Pancreas	0.204	0.051	NR	NR
Skin	0.080	0.126	0.038	0.006
Spleen	0.087	0.033	NR	NR
Stomach	0.158	0.047	0.041	0.016
Thyroid	NR	0.038	NR	NR
Uterus	NR	0.031	NR	NR
Blood	0.090	0.033	0.027	0.010

[a]Source: Tobes et al., 1983

[b]Tissue concentrations are expressed as % kg dose/g to normalize differences in animal weights.

NR = Not reported

weighing 150 g. Two rats per dose group were given 1 mg/kg methylene-labeled [^{14}C]4,4'-methylene-bis(2-chloroaniline) in arachis oil intraperitoneal or 10 mg/kg orally. Manis et al. (1984) studied the distribution of methylene-labeled [^{14}C]4,4'-methylene-bis(2-chloroaniline) in groups of four male, beagle-type mongrel dogs following intravenous or cutaneous administration of 10 mg in propylene glycol (intravenous) or acetone (cutaneous). The results of both studies are shown in Table 5-2. For all routes of administration, the liver and fat tissues showed the most radioactivity. After intravenous administration, the disappearance of radioactivity from the blood was biphasic, with a volume of distribution of 244 ℓ for the four dogs (Manis et al., 1984). Twenty-four hours after cutaneous administration, amounts of radioactivity in the tissues were 10-20 times lower than after intravenous administration; 90% of the administered radioactivity was located in the skin at the injection site.

4,4'-Methylene-bis(2-chloroaniline) has been detected within human erythrocytes (Williams, 1979). Because of their ~120-day lifetime, they may serve as storage depots for the compound.

5.3. METABOLISM

Farmer et al. (1981) (see Section 5.1.) partially identified the metabolites of [^{14}C]4,4'-methylene-bis(2-chloroaniline) in the urine of rats collected 5 days after treatment by intraperitoneal injection. Results showed that 95-97% of the radioactivity in the urine of rats given 13 or 100 mg/kg [^{14}C]4,4'-methylene-bis(2-chloroaniline) was in the form of very polar compounds, while 1-2% was tentatively identified as 4,4'-methylene-bis(2-chloroaniline). Two other less polar compounds were isolated, but not identified. Deconjugation of the very polar metabolite fraction with a sulphatase and glucuronidase mixture resulted in the isolation of several

TABLE 5-2

Tissue Distribution of Radioactivity in Rats and Dogs After Administration of [^{14}C]Methylene-bis(2-Chloroaniline)

Tissue	Percentage of Administered Dose of Radioactivity			
	Rat[a]		Dog[b]	
	Oral (10 mg/kg)	i.p. (1 mg/kg)	i.v. (10 mg)	p.c. (10 mg)
Bile	NR	NR	32	0.62
Fat	0.22	0.43	0.18	NR
Kidney	0.16	0.26	0.18	0.02
Liver	1.91	2.27	3.1	0.28
Lung	0.07	0.18	NR	NR
Skin[c]	NR	NR	NA	90
Blood	0.14	0.20	NR	NR
Feces	49.5	69.1	NR	NR
Urine	23.75	29.45	46	1.3
Total	85.75	101.89	81.46	92.22

[a]Farmer et al., 1981

[b]Manis et al., 1984

[c]Site of injection

NR = Not reported; NA = not applicable; i.p. = intraperitoneal; i.v. = intravenous; p.c. = percutaneous

metabolites, one of which was identified as 4,4'-methylene-bis(2-chloroaniline). Conjugated and unconjugated 4,4'-methylene-bis(2-chloroaniline) constituted 3-6% of urinary radioactivity. Further analysis with glucuronidase alone led the authors to conclude that the larger portion of the conjugates were sulphates. Thus, it appeared that the low dose and high dose of 4,4'-methylene-bis(2-chloroaniline) were extensively metabolized in the rat by the same metabolic systems. The rapid urinary excretion of radioactivity after treatment (Section 5.4.) suggested that metabolism was rapid. Farmer et al. (1981) also analyzed the urine of humans known to be exposed to 4,4'-methylene-bis(2-chloroaniline). Unchanged parent compound was identified at levels $\leq$1500 nmol/ℓ (0.4 mg/ℓ), but the metabolites most prevalent in rat urine were not located in the human urine. The investigators concluded that important species differences exist in the metabolism of 4,4'-methylene-bis(2-chloroaniline) by rats and humans.

Manis and Braselton (1984) identified the major 4,4'-methylene-bis-(2-chloroaniline) metabolite in the urine of dogs in the Manis et al. (1984) study as 5-hydroxy-3,3-dichloro-4,4-diaminodiphenylmethane-5-sulfate, the sulfate conjugate of a reactive intermediate.

Morton et al. (1986) investigated the formation of 4,4'-methylene-bis-(2-chloroaniline) metabolites by incubating rat or human liver microsomes with [^{14}C]4,4'-methylene-bis(2-chloroaniline) and appropriate cofactors and then extracting and analyzing the products. Results showed that the major product in both species was the n-hydroxy derivative, formed at rates of 335 and ~500 pmol/min/mg of protein in rats and humans, respectively. Pretreatment of rats with phenobarbital (but not with 3-methylcholanthrene) resulted in a 4- to 8-fold increase in the rate of formation of this metabolite. This compound induced unscheduled DNA synthesis in dog

urothelial cells. These findings support the possibility that 4,4'-methylene-bis(2-chloroaniline) is metabolized by liver enzymes to a compound that may be carcinocenic to humans. Other shared metabolites included the benzhydol and the 6-hydroxy derivatives of 4,4'-methylene-bis-(2-chloraniline). The former was formed at rates of 82 and 60 pmol/min/mg of protein and the latter at 92 and 21 pmol/min/mg of protein in rats and humans, respectively.

5.4. EXCRETION

When a worker in a 4,4'-methylene-bis(2-chloroaniline) production plant was accidentally exposed to molten 4,4'-methylene-bis(2-chloroaniline) on his chest, abdomen, and extremities, urine levels of 4,4'-methylene-bis-(2-chloroaniline) were monitored for 9 days and the half-life was determined to be ~23 hours, assuming a one-compartment model (Osorio et al., 1986). The amount of 4,4'-methylene-bis(2-chloroaniline) in the urine of a man, who was accidently exposed to the compound when hot liquid was sprayed over his face and in his mouth, was measured for 20 days (Hosein and Van Roosmalen, 1978). Data showed that the compound was excreted rapidly for the first 18 hours after exposure; urinary levels 2 weeks later were negligible.

Four female Sprague-Dawley rats were given 0.49 mg/kg [^{14}C]4,4'-methylene-bis(2-chloroaniline) intravenously. Twelve hours after dosing, 35.5% of the radioactivity had been excreted; by 24 hours, 79.4% had been excreted, and by 48 hours, >90% of the administered radioactivity had been excreted in the urine or feces. Fecal excretion predominated, accounting for 73.4% of the dose at 48 hours (Tobes et al., 1983).

Farmer et al. (1981) (see Section 5.1.) measured the excretion of radioactivity in feces and urine of rats for 48 hours after treatment with [^{14}C]4,4'-methylene-bis(2-chloroaniline) at 1 mg/kg by intraperitoneal

injection or 10 mg/kg by gavage. Fecal excretion predominated, accounting for 69.1% of the dose after intraperitoneal administration and 59.5% after oral treatment. Urinary excretion accounted for 29.5 and 23.8% after intraperitoneal and oral treatment, respectively. Urinary excretion following oral treatment was ~90% complete at 24 hours.

Manis et al. (1984) measured distribution of radioactivity in dogs 24 hours after intravenous and percutaneous administration of [^{14}C]4,4'-methylene-bis(2-chloroaniline). Following intravenous administration, 32% of the administered dose was in the gallbladder bile and 46% was in the urine, suggesting that biliary excretion may be an important route of elimination for 4,4'-methylene-bis(2-chloroaniline).

5.5. SUMMARY

Excretion data in rats indicate that 4,4'-methylene-bis(2-chloroaniline) is rapidly absorbed from the gastrointestinal tract (Farmer et al., 1981). It has been shown that 4,4'-methylene-bis(2-chloroaniline) can be absorbed through the skin of rabbits (E.I. DuPont de Nemours and Company, Inc., 1977), dogs (Manis et al., 1984) and humans (Osorio et al., 1986). In the dog study, absorption was estimated at 2.4-10% of the applied dose over a 24-hour exposure period. Absorbed 4,4'-methylene-bis(2-chloroaniline) is rapidly distributed throughout the body. Highest levels, regardless of route of administration, are located in the liver and fat, but no organ or tissue appears to preferentially accumulate or retain 4,4'-methylene-bis-(2-chloroaniline) or its metabolites.

Farmer et al. (1981) determined in rats that both low and high doses of 4,4'-methylene-bis(2-chloroaniline) were extensively metabolized by the same metabolic systems. They showed that the major urinary products were conjugates of several metabolites and that only 1-2% was excreted unchanged in

the urine. In the urine of humans with known exposure to 4,4'-methylene-bis(2-chloroaniline), however, only unmetabolized compound was identified in the urine and the investigators concluded that important species differences exist in the metabolism of the compound. In vitro studies, however, indicate that liver microsomal preparations from rats and humans biotransform 4,4'-methylene-bis(2-chloroaniline) to the N-hydroxy, 6-hydroxy and benzhydrol derivatives (Morton et al., 1986).

Based on accidental human dermal exposure to 4,4'-methylene-bis-(2-chloroaniline), the half-life in a human was estimated as ~23 hours (Osorio et at., 1986). Studies using rats (Farmer et al., 1981; Tobes et al., 1983) indicate that excretion is rapid after intravenous, intraperitoneal or oral administration. Fecal excretion exceeds urinary excretion roughly by a factor of two regardless of route of administration. A distribution dog study reported that 32% of the dose was located in the bile 24 hours after intravenous treatment, indicating that biliary excretion is important in the elimination of 4,4'-methylene-bis(2-chloroaniline).

6. EFFECTS

6.1. SYSTEMIC TOXICITY

6.1.1. Inhalation Exposure.

6.1.1.1. SUBCHRONIC -- Pertinent data regarding systemic toxicity associated with subchronic inhalation exposure of humans or animals to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

6.1.1.2. CHRONIC -- Linch et al. (1971) conducted a cohort study of 62 workers at a 4,4'-methylene-bis(2-chloroaniline) manufacturing plant [31 were exposed to 4,4'-methylene-bis(2-chloroaniline) and 31 were not exposed] to determine whether there was any evidence of chronic systemic disease in the group. The length of 4,4'-methylene-bis(2-chloroaniline) exposure time ranged from 6 months to 16 years. Although attempts were made to measure concentrations of 4,4'-methylene-bis(2-chloroaniline) vapor and dusts in the air, the results were variable and unreliable. No differences were seen regarding systemic illnesses, urinary tract pathology, deaths or work absenteeism. In another group of 178 workers who had worked with 4,4'-methylene-bis(2-chloroaniline) (but not for at least 10 years) there were no differences in general health status compared with the entire plant population. The authors concluded that no abnormal health effects in these workers could be attributed to 4,4'-methylene-bis(2-chloroaniline) exposure.

In an earlier occupational study, Mastromatteo (1965) reported that reversible hematuria had occurred in workers exposed to 4,4'-methylene-bis-(2-chloroaniline). Exposure levels, however, were not precisely quantified.

6.1.2. Oral Exposure.

6.1.2.1. SUBCHRONIC -- Pertinent data regarding systemic toxicity associated with subchronic oral exposure of humans or animals to

4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

6.1.2.2. CHRONIC -- Stula et al. (1977) administered 4,4'-methylene-bis(2-chloroaniline) (purity 90%) in gelatin capsules at 100 mg/day to a group of six female purebred beagle dogs, 3 days/week for 6 weeks and then 5 days/week for $\leq$9 years. The average daily dose, calculated from data provided by the investigators, was 7.3 mg/kg. Another group of six dogs that did not receive 4,4'-methylene-bis(2-chloroaniline) served as negative controls. Dogs were weighed weekly and clinical blood chemistry and urine parameters were analyzed regularly. After 9 years of treatment, surviving dogs were sacrificed and necropsied. Major tissues were examined for gross and microscopic lesions. There were no treatment-related effects on mortality or body weights. Toxic signs noted in treated dogs included follicular cystitis, increased GPT (which usually indicates liver injury) and liver nodular hyperplasia.

Russfield et al. (1975) administered 4,4'-methylene-bis(2-chloroaniline) in the diets to groups of Charles River CD-1 mice (25 males and 25 females/group) and male Charles River CD-1 rats (25/group) for 18 months; the mice and rats were observed for an additional 6 months. Treated animals were fed a commercial diet supplemented with concentrations of 500 and 1000 ppm (rats) or 1000 and 2000 ppm (mice). Control animals received the commercial diet without the 4,4'-methylene-bis(2-chloroaniline). After 18 months of treatment, all animals were given commercial diets without 4,4'-methylene-bis(2-chloroaniline) for 6 months. Surviving animals were sacrificed, necropsied, and major organs were subjected to histopathological examination. Animals were weighed at regular intervals during the treatment and food consumption was monitored. Data for these parameters were not

reported. Results showed that 4,4'-methylene-bis(2-chloroaniline) treatment had no effect on the survival of the rats. At the end of 18 months of treatment, 96% of the controls and 80% of the treated rats survived. At the end of 20-22 months, 55% of each group survived. At the end of the treatment, body weights of the rats in the low-dose group averaged ~50 g lower than those of controls, while body weights of the high-dose group averaged 100 g lower than those of controls; these differences persisted until the end of the study. There were no "striking differences" among treated groups of rats and controls in the incidence of noncancer gross and histopathological lesions. Survival of female mice receiving the high concentration of 4,4'-methylene-bis(2-chloroaniline) appeared affected by treatment, since 14/25 mice in this group survived the study compared with 21/25 of the low-dose group and 20/25 controls. Of the male mice in the study, 18/25 controls, 13/25 in the low-dose group and 20/25 in the high-dose group survived. No treatment-related effects on body weight gain in the mice were evident. Treated mice exhibited a lower incidence and intensity of amyloidosis than controls.

Kommineni et al. (1979) assessed the effect of 4,4'-methylene-bis-(2-chloroaniline) administered in the diets to Charles River Sprague-Dawley male rats on survival, body weight gain and hemoglobin and hematocrit parameters. Groups of 50-100 rats were given semi-purified nutritionally adequate diets containing 0, 250, 500 or 1000 ppm of 4,4'-methylene-bis-(2-chloroaniline) for 18 months, after which they received the basal diet without 4,4'-methylene-bis(2-chloroaniline) for an additional 6 months. Food consumption, body weights, hematocrit and hemoglobin measurements were made periodically, either on individual rats or on 10 rats/group. Urine samples from 10 rats were collected periodically and urine was analyzed for volume and specific gravity. All rats that died before the conclusion of

the study were autopsied. All that survived until the end of the study were sacrificed and autopsied, and gross lesions and major organs were examined microscopically. Results showed that survival time decreased as dietary amounts of 4,4'-methylene-bis(2-chloroaniline) increased. Mean survival times were 88.9, 86.6, 80.4 and 65.3 weeks for the controls to highest dose groups, respectively. Differences in survival between controls and the 500 ppm dose group, and controls and the 1000 ppm dose group were statistically significant ($p<0.01$ and $p<0.001$, respectively). The mean body weight gain of rats in the 1000 ppm group was lower than that of rats in the other groups after 8 weeks of 4,4'-methylene-bis(2-chloroaniline) administration until the end of the study. Food consumption was usually <11% different from that of control rats. Body weight gain of rats in the other groups was similar to that of the control rats throughout the study. Hematocrit and hemoglobin values were slightly less in the 1000 ppm group than in controls, but the values were within normal ranges observed in this laboratory. Noncancer results of pathological examinations were not reported.

A study by Stula et al. (1975), in which 4,4'-methylene-bis(2-chloroaniline) was given to groups of 50 male and 50 female Charles River CD rats at dietary concentrations of 0 or 1000 ppm for $\leq$2 years, reported liver changes including hepatocytomegaly, fatty change, necrosis, bile duct proliferation and fibrosis in rats that received 4,4'-methylene-bis-(2-chloroaniline). Although statistical analysis was not performed, days on test to 50% survival and the average number of days on test suggest that survival was reduced in treated rats of both sexes compared with controls.

6.1.3. Other Relevant Information. A worker in an 4,4'-methylene-bis-(2-chloroaniline) production plant was exposed to 4,4'-methylene-bis-(2-chloroaniline) when he was accidentally sprayed with molten 4,4'-methylene-bis(2-chloroaniline) while cleaning a clogged delivery line.

Exposure to the chest, abdomen and extremities occurred over several seconds. According to the report, no 4,4'-methylene-bis(2-chloroaniline) was ingested. After the 4,4'-methylene-bis(2-chloroaniline) had been gently washed from the skin, the worker was described as having slight erythema and a burning sensation. Laboratory results revealed normal renal and liver function tests, no methemoglobinemia and no protein or red blood cells in the urine. Urinary levels of 4,4'-methylene-bis(2-chloroaniline) were 1707 ppb 9 hours after exposure, but were nondetectable after 11 days. In this worker, no acute effects from the 4,4'-methylene-bis(2-chloroaniline) exposure were observed (Osorio et al., 1986).

According to Linch et al. (1971), human exposure to 4,4'-methylene-bis-(2-chloroaniline) in sufficient amounts may cause cyanogenic effects. Employees in 4,4'-methylene-bis(2-chloroaniline) production plants have had urinary levels of 4,4'-methylene-bis(2-chloroaniline) as high as 25 mg/ℓ without other observable symptoms of exposure (Linch et al., 1971).

One study reported the oral LD_{50} in male rats as 750 mg/kg (Miller and Sherman, 1965), but no other details of this study are available. Another report gave the approximate lethal dose for rats as 1000 mg/kg; signs of toxicity included polyuria, cyanosis, weakness and pallor. Doses of 200 mg/kg every day for 10 days resulted in cyanosis, pallor, growth depression and blood and urine abnormalities, but not mortality (Reinke, 1963). Salamone (1981) reported an intraperitoneal LD_{50} of 64 mg/kg for 4,4'methylene-bis(2-chloroaniline) in DMSO in mice (gender not specified) observed for $\leq$7 days after treatment.

Silk et al. (1989) conducted a study to measure the binding of 4,4'-methylene-bis(2-chloroaniline) to rat liver DNA *in vivo* and binding of

the N-hydroxylated derivative in vitro. For the in vivo experiments, male Wistar rats were given an intraperitoneal injection of radiolabeled compound in DMSO. After 24 hours, the rats were sacrificed and the livers removed for DNA extraction, isolation and purification. For in vitro experiments, rat liver slices were incubated for 2.5 hours at 37°C with radiolabeled compound. The DNA was then extracted and purified. Results showed that three DNA adducts formed when 4,4'-methylene-bis(2-chloroaniline) was injected intraperitoneal into rats or when it was incubated with rat liver slices. One of the adducts seemed to result from n-hydroxylation of 4,4'-methylene-bis(2-chloroaniline); the other two were not identified. In a study designed to compare DNA binding and DNA-adduct formation of 4,4'-methylene-bis(2-chloroaniline) in explant cultures of human and dog bladder, Stoner et al. (1987) found that 4,4'-methylene-bis(2-chloroaniline) does bind to DNA of both species. The amount of binding was related to the concentration of 4,4'-methylene-bis(2-chloroaniline) used. Of several 4,4'-methylene-bis(2-chloroaniline)-DNA adducts identified, three were found to be formed in both species.

6.2. CARCINOGENICITY

6.2.1. Inhalation. Linch et al. (1971) conducted a cohort study of 62 workers (see Section 6.1.1.2.) at a 4,4'-methylene-bis(2-chloroaniline) manufacturing plant [31 were exposed to 4,4'-methylene-bis(2-chloroaniline) and 31 were not exposed] to determine whether there was any human carcinogenic potential. The length of 4,4'-methylene-bis(2-chloroaniline) exposure time ranged from 6 months to 16 years. No difference in the incidence of malignant tumors in the two groups was observed.

NIOSH (1986, 1987) diagnosed two cases of bladder cancer (the theoretical "expected number" of bladder cancer cases is 0.39) involving nonsmoking men under 30 years of age among a cohort of 370 workers exposed

to 4,4'-methylene-bis(2-chloroaniline) who submitted to a urine cytology screening examination. The screening test revealed neither positive nor suspicious cases. The first case was identified by the examination of an individual who was diagnosed with low-grade intermittent hematuria. The diagnosis of this case provided the impetus to offer cystoscopic examination to workers with atypical cells or hematuria on the cytology examination and to an equal number of workers who had highest exposures to 4,4'-methylene-bis(2-chloroaniline). The second case was diagnosed during the cystoscopic examination of 41 workers. NIOSH (1987) expressed concern because 4,4'-methylene-bis(2-chloroaniline) is structurally similar to benzidine, which is known to cause bladder cancers in humans, and because animals exposed to 4,4'-methylene-bis(2-chloroaniline) developed bladder tumors.

6.2.2. Oral. Russfield et al. (1975) (see Section 6.1.2.2.) gave 4,4'-methylene-bis(2-chloroaniline) in the diets to groups of 25 male and 25 female Charles River CD-1 mice and 25 Charles River CD-1 male rats. Animals, 4-6 weeks old at the beginning of the study, were fed a commercial diet for 2 weeks, then were fed the commercial diet supplemented with concentrations of 500 or 1000 ppm (rats) or 1000 or 2000 ppm (mice) 4,4'-methylene-bis(2-chloroaniline) for 18 months. Control animals received the commercial diet without 4,4'-methylene-bis(2-chloroaniline). After 18 months of treatment, all animals were given commercial diets without 4,4'-methylene-bis(2-chloroaniline) for 6 months. Surviving animals were then sacrificed, necropsied and major organs examined histopathologically. Animals were scored for tumor formation at the end of the study. Of the female mice, 0/20 controls, 9/21 at the low dose and 7/14 at the high dose had hepatomas. Results are shown in Table 6-1. The differences between the numbers of hepatomas seen in the treated females compared with the numbers

TABLE 6-1

Number of tumors in Charles River CD-1 Mice Fed Diets Containing 4,4-Methylene-bis(2-chloroaniline) for 18 Months and Observed for an Additional 6 Months[a]

Sex	Concentration (ppm)	Tumor Type	Incidence of Tumors
F	0	hepatoma	0/20
	1000		9/21[b]
	2000		7/14[b]
F	0	hemangioma	0/20
	1000		0/21
	2000		4/14
F	0	hemangiosarcoma	0/20
	1000		0/21
	2000		2/14
M	0	hemangioma	0/20
	1000		2/21
	2000		5/14
M	0	hemangiosarcoma	0/20
	1000		1/21
	2000		3/14

QUALITY OF EVIDENCE

Strength of study: The compound was administered to both sexes at two dose levels and was 97% pure. Natural route of exposure; adequate duration of exposure

Weakness of study: Small number of animals per dose group; MTD may have excluded

Overall adequacy: Adequate

[a]Source: Russfield et al., 1975

[b]Significantly different from control ($p<0.01$)

seen in the controls were statistically significant ($p<0.01$). There also appeared to be an increased incidence of hemangiomas and hemangiosarcomas in treated mice, compared with controls. While the numbers of vascular tumors observed in male and female treated mice were greater than those in the controls, they were not greater than those observed in historical controls and were not considered significant by the authors. Mice that received 4,4'-methylene-bis(2-chloroaniline) in their diets had smaller incidences of amyloid tumors than did controls (14/38 controls, 7/34 low dose, 4/34 high dose); no explanation for this decrease was given (Russfield et al, 1975). Of the rats that survived, 0/22 controls, 1/22 at the low dose, and 4/19 at the high dose had hepatomas; 0/22 controls, 3/22 at the low dose and 4/19 at the high dose had adenomatosis of the lung. The authors indicated that the difference between the incidence of tumors in the treated groups and that of controls was not statistically significant. Other neoplasms seen in treated rats but not the controls included a tumor of the ear duct and adenocarcinomas of the lung, stomach, small intestine and urinary bladder.

Stula et al. (1975) studied the effects of 4,4'-methylene-bis(2-chloroaniline) (~95% pure) on tumor formation in groups of 50 male and 50 female Charles River CD rats when given at concentrations of 0 or 1000 ppm in the diet for $\leq$2 years. After 1 year of treatment, 6 rats/group were sacrificed and necropsied. All rats were necropsied, either at time of death during the study or at terminal sacrifice, and 30 organs were sampled for histological examination. Results (Table 6-2) showed that treated rats had a higher incidence of lung adenocarcinoma than controls. Adenomatosis was observed after 1 year of treatment. In rare cases, a pleural biphasic tumor accompanied the lung tumors. The incidence of the lung adenocarcinomas was statistically significant ($p<0.05$) compared with controls in both sexes.

TABLE 6-2

Incidence of Tumors in Charles River CD Rats Fed 4,4-Methylene-bis-(2-Chloroaniline) in the Diet for ≤2 Years*

Sex	Concentration (ppm)	Tumor Type	Incidence of Tumors	p Value
F	0	lung adenocarcinoma	0/44	NA
F	1000		21/44	$p<0.05$
M	0		0/44	NA
M	1000		27/44	$p<0.05$

QUALITY OF EVIDENCE

Strengths of Study: Chemical 95% pure, adequate number of animals per group; natural route of exposure, adequate duration of exposure, MTD reached

Weakness of Study: Compound administered at only one dose level

Overall Adequacy: Adequate

*Source: Stula et al., 1975

NA = Not applicable

Liver tumors were also observed in treated rats of both sexes at incidences greater than in controls; however, the differences were not statistically significant. The incidence of other types of tumors in the treated rats was not different from the incidence in control rats.

A study by Kommineni et al. (1979) assessed the effect of 4,4'-methylene-bis(2-chloroaniline) given orally on tumor formation in Charles River Sprague-Dawley male rats. The rats, 5 weeks old, were divided into four treatment groups consisting of 50-100 animals. Each group received different dietary amounts of 4,4'-methylene-bis(2-chloroaniline). Dietary concentrations of 0, 250, 500 or 1000 ppm of 4,4'-methylene-bis(2-chloroaniline) were given to the rats for 18 months. After this time, the regular diet without 4,4'-methylene-bis(2-chloroaniline) was fed for an additional 6 months. Every 4 weeks, individual rats were scored for size and location of palpable masses. All rats that died before the conclusion of the study were autopsied. All that survived until the end of the study were sacrificed and autopsied. Gross lesions and major organs were examined microscopically.

Results of the carcinogenicity study are shown in Table 6-3. Increased incidences of pulmonary adenomas and adenocarcinomas, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas were seen in a dose-related fashion and were attributed to administration of 4,4'-methylene-bis(2-chloroaniline). Metastasis of these neoplasms to other organs, such as kidneys, pituitary gland and pancreas, was also noted.

Stula et al. (1977) administered 4,4'-methyelne-bis(2-chloroaniline) (90% pure) at 100 mg/day in gelatin capsules to a group of six female purebred beagle dogs on 3 days/week for 6 weeks and then on 5 days/week for $\leq$9 years (average daily dose, 7.3 mg/kg). Another group of six dogs that did not receive 4,4'-methylene-bis(2-chloroaniline) served as controls.

TABLE 6-3

Incidence of Tumors in Male Charles River Sprague-Dawley Rats Fed Diets Containing 4,4-Methylene-bis-(2-Chloroaniline) for 18 Months then Observed for 5 Additional Months[a]

Concentration (ppm)	Tumor Type	Incidence[b] (%)	Incidence[c] (number bearing tumors/ number necropsied)	p Value (compared with control)
0	lung adenocarinomas[d]	0	0/100	NA
250		14	14/100	$p \leq 0.001$
500		17	20/75	$p \leq 0.001$
1000		62	31/50	$p \leq 0.001$
0	all primary lung	1	1/100	NA
250	neoplasms	23	23/100	$p \leq 0.001$
500		37	28/75	$p \leq 0.001$
1000		70	35/50	$p \leq 0.001$
0	mammary adenocarcinomas	1	1/100	NA
250		5	5/100	NS
500		11	8/75	$p \leq 0.01$ and >0.001
1000		28	14/50	$p \leq 0.001$
0	Zymbal gland carcinomas	1	1/100	NA
250		8	8/100	$p \leq 0.05$ and >0.01
500		7	5/75	NS
1000		22	11/50	$p \leq 0.001$
0	hepatocellular carcinomas	0	0/100	NA
250		3	3/100	NS
500		4	3/75	NS
1000		36	18/50	$p \leq 0.001$

QUALITY OF EVIDENCE

Strengths of study: The compound was administered at three dose levels. Adequate numbers of animals per group; natural route of exposure; adequate duration of exposure, adequate survival, MTD reached

Weakness of study: Compound administered to male rats only; purity of compound not reported

Overall adequacy: Adequate

[a]Source: Kommineni et al., 1979

[b]Data provided by investigators

[c]Obtained by multiplying incidence data expressed as percent provided by investigators by the number necropsied and rounding to nearest integer

[d]Includes bronchiolar-alveolar cell carcinomas

NA = Not applicable; NS = not stated

After 8.3 or 9 years of treatment, five surviving dogs of the treated group and all control dogs were killed and necropsied. Major tissues were subjected to histopathological examination. Results are shown in Table 6-4. After 8.3-9 years of treatment, four of five treated dogs, but no controls, had carcinomas of the urinary bladder.

6.2.3. Other Relevant Information. A group of 25 male and 25 female Wistar rats was given a low protein diet containing 0.1% 4,4'-methylene-bis-(2-chloroaniline) for 500 days; a control group of rats was given a similar diet without 4,4'-methylene-bis(2-chloroaniline) (Grundmann and Steinhoff, 1970; Steinhoff and Grundmann, 1971). After 500 days, all rats received the low-protein diet without 4,4'-methylene-bis(2-chloroaniline) for the remainder of their lives. The cumulative dose in the treated group was estimated at 27 g/kg. Among treated rats, 23 males and 20 females died with tumors; 22 and 18, respectively, were liver tumors. Thirteen treated rats (8 males, 5 females) had primary lung tumors and 10 of these also had hepatomas. Two mammary adenomas were additionally reported in the group. The average survival times were 730, 565 and 535 days for controls, treated males and females, respectively.

When 94% pure 4,4'-methylene-bis(2-chloroaniline) as a suspension (vehicle not reported) was given to 17 male and 17 female Wistar rats by weekly subcutaneous injections of 500 or 1000 mg/kg (total dose of 25 g/kg bw), 9 rats developed liver cell carcinomas and 7 developed primary lung carcinomas; 22 rats died with a total of 29 malignant tumors. Thirteen malignant tumors, including 1 lung tumor, developed in 50 control rats that survived $\leq$1040 days. No malignant tumors of the liver were seen (Steinhoff and Grundmann, 1971).

TABLE 6-4

Incidence of Tumors in Female Beagle Dogs Given Capsules Containing 4,4-Methylene-bis(2-chloroaniline) for ≤9 Years[a]

Dose (mg/kg/day)	Tumor Type	Tumor Incidence
0	urinary bladder carcinoma	0/6
7.3[b]		4/5

QUALITY OF EVIDENCE

Strengths of Study: Chemical was 90% pure; natural route of exposure; adequate duration of exposure

Weakness of Study: Compound administered at one dose level; only female animals used; small number of animals

Overall Adequacy: Inadequate

[a]Source: Stula et al., 1977

[b]Time weighted, expanded average dose for dogs given 100 mg capsule 3 days/week for 6 weeks, then 5 days/week for 9 years (calculated from data provided by investigators)

6.3. MUTAGENICITY

Results of mutagenicity testing of 4,4'-methylene-bis(2-chloroaniline) are shown in Table 6-5. It has been reported to be positive in the following bacterial and fungi tests: (1) in reverse mutation assays with Salmonella typhimurium strains TA98 and TA100, with and without microsomal activation (Takemura and Shimizu, 1978; Ho et al., 1979; Shimizu et al., 1982; McCann et al., 1975; Brooks and Dean, 1981); (2) in a reverse mutation test with Escherichia coli (Matsushima et al., 1981); (3) in a phage induction test with E. coli (Thomson, 1981); (4) in an E. coli DNA repair assay with microsomal activation (Ichinotsubo et al., 1981); (5) in a rec-assay with Bacillus subtilis (Kada, 1981); (6) in mitotic gene conversion and mitotic aneuploidy tests with Saccharomyces cerevisiae (Sharp and Parry, 1981; Parry and Sharp, 1981); and (7) in a yeast mutation assay with and without activation (Ho et al., 1979). It has given mixed responses in assays with cells from more complex organisms. It was positive in cell transformation assays with Syrian hamster embryo and Syrian hamster kidney cells (Casto, 1980; Purchase et al., 1978) and Balb/3T3 cells (Dunkel et al., 1981). It failed to induce sex-linked recessive lethal mutations in Drosophila melanogaster (Ho et al., 1979). It did not produce chromosomal aberrations or sister chromatid exchanges (SCE) in Chinese hamster ovary cells or human leucocytes (Galloway et al., 1985; Ho et al., 1979). It was reported to produce unscheduled DNA synthesis (UDS) in rodent and rabbit hepatocytes (McQueen et al., 1981, 1983; McQueen and Williams, 1982; Mori et al., 1988).

In comparative studies with hamster embryo cells and human male embryo lung cells (Casto, 1983), hamster cells were more sensitive to the lethal effects of 4,4'-methylene-bis(2-chloroaniline), the LD_{50} values (µg/mℓ) being 45 and 270, respectively, after 2 hours of treatment.

TABLE 6-5

Genotoxicity Testing of 4,4'-Methylene-bis(2-chloroaniline)

Assay	Indicator/Organism	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA98 TA100	NR	NR	S-9	 - +	NC	Takemura and Shimizu, 1978
Reverse mutation	S. typhimurium (strain not specified)	NR	NR	microsomal	+	NC	Ho et al., 1979
Reverse mutation	S. typhimurium TA1535 TA1537 TA1538 TA98 TA100 TA92	preincubation plate incorporation	0.2-2000 µg/plate	 ±S-9 ±S-9 ±S-9 ±S-9 ±S-9 ±S-9	 ± ± ± + + ±	Vehicle probably DMSO; lowest effective dose not reported	Brooks and Dean, 1981
Reverse mutation	S. typhimurium TA98	NR	10-1000 µg/plate	±S-9	- +	0.53 reversions/µg	Shimizu et al., 1982
	TA100			±S-9	- +	2.74 reversions/µg	
Reverse mutation	S. typhimurium TA100	plate incorporation	NR	none	+	2.7 reversions/µg	McCann et al., 1975
DNA repair	Escherichia coli rec⁻	spot test	NR	S-9	+	NC	Ichinotsubo et al., 1981
Reverse mutation	E. coli WP28/r WP2uvrA WP2uvrA/pKM101	preincubation plate incorporation	NR	 ±S-9 ±S-9 ±S-9	 -/NT ± ±	Vehicle was DMSO	Matsushima et al., 1981
Prophage induction	E. coli Lambda-sensitive	liquid suspension	1000 or 2000 µg/mL	±S-9	+	Vehicle was DMSO	Thomson, 1981
Rec-assay for DNA damage	Bacillus subtilis H17rec⁺ and M45rec⁻	filter disc test	1 mg/disc	±S-9 ±S-9	+	S-9 harvested from livers of rats, yellowtail fish and/or Japanese clams; tested in spores rather than negative stage	Kada, 1981

TABLE 6-5 (cont.)

Assay	Indicator/Organism	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Yeast mutation	NR	NR	NR	none microsomal	+ +	NC	Ho et al., 1979
Mitotic gene conversion	Saccharomyces cerevisiae JD1	preincubation plate incorporation	10 µg	S-9	+	Vehicle was DMSO; concentration was LED	Sharp and Parry, 1981
Mitotic aneuploidy	S. cerevisiae D6	preincubation plate incorporation	5 µg	S-9	+	Concentration was LED	Parry and Sharp, 1981
Sex-linked recessive lethal	Drosophila melanogaster	NR	NR	NA	-	NC	Ho et al., 1979
Unscheduled DNA synthesis	rabbit hepatocytes	18-hour incubation	10^{-5} to 10^{-4} M	NA	+	Weak response	McQueen et al., 1983
Unscheduled DNA synthesis	rat hepatocytes	20-hour incubation	10^{-5} to 10^{-4} M	NA	+	NC	Mori et al., 1988
Unscheduled DNA synthesis	rat hepatocytes	18-hour incubation	10^{-6} to $5x10^{-4}$ M	NA	+	NC	McQueen et al., 1981
Unscheduled DNA synthesis	mouse hepatocytes	18-hour incubation	10^{-6} to $5x10^{-4}$ M	NA	+	NC	McQueen et al., 1981
Unscheduled DNA synthesis	hamster hepatocytes	18-hour incubation	10^{-6} to $5x10^{-4}$ M	NA	+	NC	McQueen et al., 1981
Unscheduled DNA synthesis and cytotoxicity	rat heapatocytes	2- to 18-hour incubation	10^{-4} to 10^{-3} M	NA	+	Cells monitored for release of LDH and GOT	McQueen and Williams, 1982
Chromosomal aberrations SCE	human leucocytes	NR	NR	- +	- -	NC	Ho et al., 1979
SCE	Chinese hamster ovary	26-hour liquid incubation	NR	- S-9	- -	NC	Galloway et al., 1985
Cell transformation	Syrian hamster embryo/SA7	2- or 18-hour liquid incubation	5-20 µg/ml	NA	+	NC	Casto, 1980

TABLE 6-5 (cont.)

Assay	Indicator/Organism	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Cell transformation	Syrian hamster kidney	liquid incubation	NR	S-9	+	NC	Purchase et al., 1978
Cell transformation	Balb/3T3	3-day liquid incubation	0.01-1.0 µg/mL	NA	+	NC	Dunkel et al., 1981

NR = Not reported; NC = no comment; NT = not tested

Hamster cells were also more sensitive to DNA breakage by 4,4'-methylene-bis(2-chloroaniline) by the same order of magnitude as that for the LD_{50} values.

6.4. DEVELOPMENTAL TOXICITY

Pertinent data regarding the developmental toxicity of 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

6.5. REPRODUCTIVE TOXICITY

Pertinent data regarding the other reproductive effects of 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

6.6. SUMMARY

The carcinogenic effects of 4,4'-methylene-bis(2-chloroaniline) administered in the diets of mice, rats and dogs have been the subject of several studies. Results have shown that administration in the diet to CD-1 mice produced increased incidences of hepatomas in females compared with controls (Russfield et al., 1975) (see Table 6-1). When given in the diet to male and female Charles River CD rats, 4,4'-methylene-bis(2-chloroaniline) produced statistically significant increased incidences of lung adenocarcinomas compared with controls (Stula et al., 1975) (see Table 6-2). Kommineni et al. (1979) reported that dietary 4,4'-methylene-bis(2-chloroaniline) given to male Charles River Sprague-Dawley rats for 18 months followed by 6 months of observation led to increased incidences of pulmonary adenomas and adenocarcinomas, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas compared with controls (see Table 6-3). Four of five female beagle dogs given 4,4'-methylene-bis(2-chloroaniline) in gelatin capsules for $\leq$9 years developed urinary bladder tumors while no control dogs developed such tumors (Stula et al., 1977) (see Table 6-4).

4,4'-Methylene-bis(2-chloroaniline) has been shown to be mutagenic in the Salmonella reverse mutation assay, with and without microsomal activation (Takemura and Shimizu, 1978; Ho et al., 1979; Shimizu et al., 1982; McCann et al., 1975), in an E. coli DNA repair assay with microsomal activation (Ichinotsubo et al., 1981) and in a yeast mutation assay with and without activation (Ho et al., 1979). It failed to induce sex-linked recessive lethal mutations in Drosophila melanogaster (Ho et al., 1979), and it did not produce chromatid aberrations or SCE in Chinese hamster ovary cells or human leucocytes (Galloway et al., 1985; Ho et al., 1979), (see Table 6-5).

Occupational exposure to 4,4'-methylene-bis(2-chloroaniline) has been associated with a reversible form of hematuria, but exposures were not precisely quantified (Mastromatteo, 1965). Signs of systemic toxicity, which was due to oral administration of 4,4'-methylene-bis(2-chloroaniline) in animals, included liver injury in female beagle dogs administered 7.3 mg/kg/day for 9 years (Stula et al., 1977), high mortality in female CD-1 mice at dietary concentrations of 2000 ppm for 18 months followed by 6 months on a normal diet (Russfield et al., 1975), decreased body weight gains in CD-1 male rats administered 500 or 1000 ppm for 18 months followed by 6 months of observation (Russfield et al., 1975), decreased survival time in CR Sprague-Dawley rats administered 4,4'-methylene-bis(2-chloroaniline) in the diet at 0, 250, 500, and 1000 ppm for 18 months and observed for 6 months (Kommineni et al., 1979), and liver effects including hepatocytomegaly, fatty change, necrosis, bile duct proliferation and fibrosis in Charles River CD rats administered 1000 ppm in the diets for $\leq$2 years (Stula et al., 1975).

In vitro studies with rat liver cells (Silk et al., 1989), and dog and human bladder explant cultures (Stoner et al., 1987) have led to the isolation of 4,4'-methylene-bis(2-chloroaniline)-DNA adducts, suggesting that 4,4'-methylene-bis(2-chloroaniline) can bind to DNA in these species. Three of the adducts were common to dog and human cell cultures.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

The ACGIH (1988) adopted a TLV-TWA of 0.02 ppm (~0.22 mg/m³). Provided that skin contact is avoided, this value will probably prevent systemic poisoning (ACGIH, 1986). Because 4,4'-methylene-bis(2-chloroaniline) may cause cancer in humans, probably in the liver or bladder, 4,4'-methylene-bis(2-chloroaniline) is also designated an industrial substance suspect of carcinogenic potential to humans. OSHA (1989) established an 8-hour TWA of 0.02 ppm with a skin notation as a final rule for occupational exposure to 4,4'-methylene-bis(2-chloroaniline). This regulation is intended to protect against material health impairments and bladder cancer.

It was recommended that 4,4'-methylene-bis(2-chloroaniline) exposure to employees in the workplace be limited to 3 µg/m³ determined as a TWA concentration for a $\leq$10-hour workshift, 40-hour workweek, over a working lifetime (NIOSH, 1978).

7.2. AQUATIC

Guidelines and standards for the protection of aquatic life from exposure to 4,4-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A.

8. RISK ASSESSMENT

8.1. CARCINOGENICITY

8.1.1. Inhalation. Linch et al. (1971) reported no increase in the incidence of tumors in a cohort of 31 workers exposed to 4,4'-methylene-bis-(2-chloroaniline) in a manufacturing plant compared with 31 nonexposed workers in the same plant. NIOSH (1986, 1987), however, located two cases of tumor of the urinary bladder in preliminary studies of a cohort of 370 exposed workers who were screened with a urine cytology examination. The tumors were not diagnosed by the cytologic examination but by cystoscopy performed because of intermittent hematuria (the first case) or because of high exposure (the second case).

8.1.2. Oral. Russfield et al. (1975) administered 4,4'-methylene-bis-(2-chloroaniline) in the diets to Charles River CD-1 mice of both sexes and Charles River CD-1 male rats. Groups of 25 rats or 25 male and 25 female mice were administered a commercial diet supplemented with concentrations of 500 and 1000 ppm (rats) or 1000 and 2000 ppm (mice) 4,4'-methylene-bis-(2-chloroaniline). Results (see Table 6-1) indicate that treated female mice had a statistically increased incidence of hepatomas, compared with controls. The authors indicated that the difference between the incidence of tumors in the treated groups of rats and the incidence of tumors in the controls was not statistically significant.

Stula et al. (1975) studied the effects of 4,4'-methylene-bis(2-chloroaniline) on tumor formation in groups of 50 male and 50 female Charles River CD rats when administered at concentrations of 0 or 1000 ppm in the diet for $\leq$2 years. Results (see Table 6-2) showed that treated rats had a significantly higher incidence of lung adenocarcinomas than controls. Liver tumors were also observed in treated rats of both sexes at incidences

greater than in controls; however, th[illegible]erence was not statistically significant. A study by Kommineni et [illegible] (1979) assessed the effect of dietary 4,4'-methylene-bis(2-chloroaniline) on tumor formation in Charles River Sprague-Dawley male rats. Increased incidences of pulmonary adenocarcinomas, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas were seen (see Table 6-3); they were attributed to administration of 4,4'-methylene-bis(2-chloroaniline). Stula et al. (1977) administered 4,4'-methylene-bis(2-chloroaniline) in gelatin capsules to a group of 6 female purebred beagle dogs, 3 days/week for 6 weeks and then 5 days/week for $\leq$9 years and reported that 4/5 treated dogs, but none of the controls, had carcinomas of the urinary bladder (see Table 6-4).

8.1.3. Other Routes. 4,4'-Methylene-bis(2-chloroaniline) in saline administered to rats by subcutaneous injections was associated with the development of liver cell carcinomas and primary lung carcinomas (Steinhoff and Grundmann, 1971).

8.1.4. Weight of Evidence. The small cohort study by Linch et al. (1971) and the preliminary reports by NIOSH (1986, 1987) were lacking sufficient data to evaluate the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) to humans. Sufficient evidence exists regarding the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) when administered orally to rats, mice and dogs, and when administered subcutaneously to rats. According to the U.S. EPA (1986b) classification scheme, 4,4'-methylene-bis(2-chloroaniline) can be placed in U.S. EPA Group B2: probable carcinogenicity for humans.

8.1.5. Quantitative Risk Estimates.

8.1.5.1. INHALATION -- Pertinent data were not located regarding the carcinogenicity of inhalation exposure of animals to 4,4'-methylene-bis-(2-chloroaniline). The occupational studies by NIOSH (1986, 1987) and Linch

et al. (1971) are not useful for cancer risk assessment. Dietary studies have reported the development of liver tumors in female mice and male rats (Russfield et al., 1975; Kommineni et al., 1979), lung tumors in male and female rats (Stula et al., 1975; Kommineni et al., 1979) and urinary bladder tumors in female beagle dogs (Stula et al., 1977). Liver and lung tumors have been reported in rats following subcutaneous administration (Steinhoff and Grundmann, 1971). These data suggest that 4,4'-methylene-bis(2-chloroaniline) may be carcinogenic by any route of exposure, provided that distribution of the proximate carcinogen to the liver, lung and bladder occurs. Pharmacokinetic data (Section 5.1.) indicate that distribution to these organs does occur. Thus, it is appropriate to adopt the q_1^* of 1.3×10^{-1} $(mg/kg/day)^{-1}$ derived for oral exposure (see below) as the q_1^* for inhalation exposure as well.

In estimating the concentration of 4,4'-methylene-bis(2-chloroaniline) in air associated with specific levels of increased risk of cancer, the ratio of the extent of absorption from the respiratory tract to that from the gastrointestinal tract must be adjusted. Pharmacokinetic data suggest that absorption from the gastrointestinal tract is rapid and complete. Data are lacking regarding the extent of absorption from the respiratory tract. A default value of 50% is assumed, resulting in a respiratory:gastrointestinal absorption ratio of 0.5. By applying the adjustment factor of 0.5 and by assuming that humans weigh 70 kg and inhale 20 m^3/day, it is estimated that an air concentration of 5.4×10^{-4} mg/m^3 would be associated with an increased cancer risk of 1×10^{-5}. Concentrations in air of 5.4×10^{-5} mg/m^3 and 5.4×10^{-6} mg/m^3 are associated with increased cancer risks of 1×10^{-6} and 1×10^{-7}, respectively.

8.1.5.2. ORAL -- Russfield et al. (1975) reported a statistically significant increase in the incidence of [illegible]omas in female Charles River CD-1 mice fed diets containing 4,4'-methylene-bis(2-chloroaniline) for 18 months compared with controls. Stula et al. (1975) observed statistically significant increases compared with controls in the incidence of lung adenocarcinomas in male and female Charles River CD rats fed 4,4'-methylene-bis-(2-chloroaniline) in the diet for $\leq$2 years. Kommineni et al. (1979) reported that the incidences of lung adenocarcinomas and all primary neoplasms were significantly increased in Charles River Sprague-Dawley rats that were administered 4,4'-methylene-bis(2-chloroaniline) in the diet for 18 months. Slope factors for oral exposure to 4,4'-methylene-bis(2-chloroaniline) were determined from data from each of these studies using the GLOBAL86 program for the multistage model designed by Howe et al (1986). The data and calculations for the derivations are presented in Appendix B. A human q_1^* of 8.9×10^{-2} $(mg/kg/day)^{-1}$ was calculated from the Russfield et al. (1975) mouse study; a human q_1^* of 1.2×10^{-1} $(mg/kg/day)^{-1}$ was calculated from the Stula et al. (1975) report and a human q_1^* of 1.5×10^{-1} $(mg/kg/day)^{-1}$ was determined from the Kommineni et al. (1979) study. The q_1^* values for lung tumors in the two rat studies are similar. Because these studies used larger numbers of animals than the mouse study and early mortality was lower in the two rat studies than in the mouse study, it is more appropriate to use the rat studies for estimation of cancer potency. The most appropriate risk estimate for 4,4'-methylene-bis-(2-chloroaniline) is derived by computing the geometric average of the two q_1^* values for lung tumors in rats. The geometric mean of 0.12 and 0.15 is 0.13, the human q_1^* for oral exposure to 4,4'-methylene-bis(2-chloroaniline) is, therefore, calculated as 1.3×10^{-1} $(mg/kg/day)^{-1}$. From the

q_1^* of 1.3×10^{-1} $(mg/kg/day)^{-1}$, it is estimated that a concentration of 2.7×10^{-3} mg/ℓ in drinking water is associated with increased cancer risk to humans of 1×10^{-5}. This estimate is based on the assumption that humans weigh 70 kg and drink 2 ℓ water/day. Drinking water concentrations of 2.7×10^{-4} and 2.7×10^{-5} mg/ℓ are associated with increased cancer risks of 1×10^{-6} and 1×10^{-7}, respectively.

8.2. SYSTEMIC TOXICITY

8.2.1. Inhalation Exposure.

8.2.1.1. LESS THAN LIFETIME (SUBCHRONIC) -- Pertinent data regarding the toxicity of subchronic inhalation exposure to 4,4'-methylene-bis-(2-chloroaniline) were not located in the available literature cited in Appendix A; data are insufficient for derivation of an RfD for subchronic inhalation exposure.

8.2.1.2. CHRONIC -- Linch et al. (1971) reported no health effects on workers exposed to 4,4'-methylene-bis(2-chloroaniline). Mastromatteo (1965), however, associated occupational exposure with hematuria. Exposures were not sufficiently quantified in either study for use in derivation of an RfD for chronic inhalation exposure.

8.2.2. Oral Exposure.

8.2.2.1. LESS THAN LIFETIME (SUBCHRONIC) -- Pertinent data regarding toxicity of subchronic oral exposure to 4,4'-methylene-bis(2-chloroaniline) were not located in the available literature cited in Appendix A. Data are available for derivation of a chronic RfD and this value can be adopted as sufficiently protective for subchronic exposure. The value of 0.0007 mg/kg/day is adopted as the RfD for subchronic oral exposure to 4,4'-methylene-bis(2-chloroaniline). The confidence in this value is low.

8.2.2.2. CHRONIC -- Data from four different chronic studies using three different species are available for the determination of a chronic oral RfD for 4,4'-methylene-bis(2-chloroaniline). Stula et al. (1977) (Rec. #1, Appendix D) reported liver nodular hyperplasia, increased GPT activity and follicular cystitis in female beagle dogs (n=6) that received an average dose of 7.3 mg 4,4'-methylene-bis(2-chloroaniline)/kg/day in gelatin capsules for 9 years. These effects indicated liver injury and bladder inflammation in the dogs. There appeared to be no treatment-related effects on mortality and body weights. The weaknesses of the study are that only female dogs were used, only six dogs were given the compound and because only one dose was administered; therefore, no dose-response relationships could be determined.

Charles River CD-1 mice were given 0, 1000 (Rec. #2, Appendix D) or 2000 ppm (Rec. #3, Appendix D) for 18 months and observed for an additional 6 months in a study by Russfield et al. (1975). Assuming a food factor of 0.13 kg food/kg bw/day, these dietary concentrations correspond to doses of 0, 130 and 260 mg/kg/day, respectively. The only adverse effect observed was increased mortality in the female mice at the higher dose. The authors reported that the incidence and intensity of amyloidosis was reduced in treated mice compared with controls. These investigators also fed rats diets containing 0, 500 (Rec. #6, Appendix D) or 1000 ppm for 18 months and observed them for an additional 6 months. Assuming a food factor for rats of 0.05 kg food/kg bw/day (U.S. EPA, 1980), these dietary concentrations correspond to doses of 0, 25 and 50 mg/kg/day. Treated rats exhibited decreased mean body weights compared with controls throughout the study. This effect was dose-related. There were no effects on mortality. The Russfield et al. (1975) study using mice and rats is not considered further

for risk assessment because a 6-month recovery period was permitted after exposure, during which reversible adverse effects could have been repaired, thereby escaping detection at necropsy and histopathological examination.

Kommineni et al. (1979) fed male rats diets containing 0, 250 (Rec. #4, Appendix D), 500 (Rec. #5, Appendix D) or 1000 ppm in the diets for 18 months. They were then observed for an additional 6 months. As calculated above, corresponding doses can be estimated at 0, 12.5, 25 and 50 mg/kg/day, respectively. Significantly reduced survival was reported at 500 and 1000 ppm. Body weight gain was lower than controls. The posttreatment recovery period precludes considering this study further for risk assessment.

Stula et al. (1975) reported hepatocytomegaly, fatty liver change, liver necrosis, bile duct proliferation and fibrosis in rats fed a diet containing 1000 ppm for 2 years (Rec. #7, Appendix D). Survival also appeared to be reduced in treated rats of both sexes. The 1000 ppm level (corresponding to 50 mg/kg/day as computed above) is considered a FEL and cannot be used in risk assessment.

Comparison of the studies indicates that dogs are more sensitive to the systemic effects of 4,4'-methylene-bis(2-chloroaniline) than rats or mice because they exhibit adverse effects at lower dose levels. It is possible that adverse effects would have been identified in rats and mice at lower levels if a 6-month recovery period had not been provided. The lowest dose at which an adverse effect was noted was 7.3 mg/kg/day in female beagle dogs (Stula et al., 1977), which is considered to be a LOAEL for systemic toxicity (Rec. #1, Appendix D). The RfD for systemic toxicity is calculated by dividing the LOAEL by an uncertainty factor of 10,000 (10 to reflect the deficiencies of the data base, 10 to extrapolate from dogs to humans, 10 to provide additional protection for more sensitive individuals and 10 for estimation of a NOAEL from a LOAEL) and is 0.0007 mg/kg/day.

Confidence in the key study is low because group sizes were small, dogs of only one sex were used and only one dose was given, which precluded locating the threshold for adverse effects. Confidence in the data base is low. The other chronic studies available in rats and mice provide limited information because of the recovery period after exposure (Russfield et al.,1975; Kommineni et al., 1979) or because only one dose was given (Stula et al., 1975). Furthermore, there is no information regarding the reproductive or developmental effects of 4,4'-methylene-bis(2-chloroaniline). Confidence in the RfD, therefore, is low.

9. REPORTABLE QUANTITIES

9.1. BASED ON SYSTEMIC TOXICITY

The systemic toxicity of 4,4'-methylene-bis(2-chloroaniline) was discussed in Chapter 6. For each study considered for computation of candidate CSs, the lowest doses associated with the effects reported are summarized in Table 9-1. Effects noted in chronic exposure studies using female dogs included liver injury and urinary bladder inflammation (Stula et al., 1977); the effect seen in a chronic study with female mice was increased mortality compared with controls (Russfield et al., 1975); effects observed in male rats included dose-related decreases in body weights (Russfield et al., 1975) and decreased body weights and a dose-related decrease in mean survival times (Kommineni et al., 1979); effects reported in rats of both sexes were decreased survival and signs of liver injury, including hepatocytomegaly, fatty change, necrosis, bile duct proliferation and fibrosis (Stula et al., 1975). More severe effects may have been identified in the studies by Russfield et al. (1975) and Kommineni et al. (1979) had the rats and mice been examined at the termination of the exposure period rather than after a 6-month recovery period.

Table 9-2 presents candidate CSs for the effects presented in Table 9-1. CSs are calculated only for the lowest human equivalent dose associated with each effect in Table 9-1. The highest CS, 17.86, was calculated for significantly increased mortality compared with controls in male rats exposed to a dietary concentration of 500 ppm for 18 months (Kommineni et al., 1979). The CS of 17.86 corresponding to an RQ of 1000 is chosen to represent the chronic (noncancer) toxicity of 4,4'-methylene-bis(2-chloroaniline) (Table 9-3).

TABLE 9-1

Oral Toxicity Summary for 4,4'-Methylene-bis(2-Chloroaniline)

Species/Strain	Sex	No. at Start	Average Weight (kg)	Vehicle/ Physical State	Purity	Exposure	Transformed Animal Dose (mg/kg/day)	Equivalent Human Dose[a] (mg/kg/day)	Response	Reference
Dog/beagle	F	6	10.4[b]	dietary/ capsule	90%	100 mg/day, 3 days/week for 6 weeks, then 5 days/week for 9 years (avg. dose = 7.3 mg/kg/day)	7.3	3.87	Liver injury, bladder inflammation	Stula et al., 1977
Mouse/Charles River CD-1	F	25	0.03[c]	dietary	97%	2000 ppm for 18 months	260.0[d]	19.60	Increased mortality compared with controls	Russfield et al., 1975
Rat/Charles River CD-1	M	25	0.35[c]	dietary	97%	500 ppm for 18 months	25.0[d]	4.27	Decreased mean body weights compared with controls	Russfield et al.
Rat/Sprague-Dawley	M	100	0.35[c]	dietary	NR	500 ppm for 18 months	25.0[d]	4.27	Increased mortality compared with controls ($p<0.01$)	K[illegible] et al., 1979
Rat/Charles River CD	M/F	50/sex	0.25[c]	dietary	95%	1000 ppm for 2 years	50.0[d]	7.64	Decreased survival; liver injury	Stula et al., 1975

[a]Calculation: transformed animal dosage (mg/kg/day) x [animal body weight (kg)/reference human body weight (70 kg)]$^{1/3}$

[b]Estiamted from data provided by investigators

[c]Reference body weight (U.S. EPA, 1980)

[d]Food factors used: rats, 0.05 kg food/kg bw/day; mice, 0.13 kg food/kg bw/day (U.S. EPA, 1980)

NR = Not reported

TABLE 9-2

Composite Scores for 4,4'-Methylene-bis(2-Chloroaniline)

Species	Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS*	RQ	Reference
Dog	7.3	270.65	1.85	Liver injury; bladder inflammation	5	9.26	1000	Stula et al., 1977
Rat	25.0	299.25	1.79	Decreased mean body weights compared with controls	4	7.14	1000	Russfield et al., 1975
Rat	25.0	299.25	1.79	Increased mortality compared with controls	10	17.86	1000	Kommineni et al., 1979

*Although Equivalent Human Dose in Table 9-1 and chronic human MED, RV_d and CS in Table 9-2 are written as two digits past the decimal, no rounding was performed in the chain of calculations starting with transformed animal dose and ending with CS.

TABLE 9-3

4,4'-Methylene-bis(2-Chloroaniline)

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral, diet
Species/Sex:	rat/male
Dose*:	299.25 mg/day
Duration:	18 months
Effect:	increased mortality compared with controls
RV_d:	1.79
RV_e:	10
CS:	17.86
RQ:	1000
Reference:	Kommineni et al., 1979

*Equivalent human dose

9.2. BASED ON CARCINOGENICITY

The carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) was discussed in Chapter 6. In the only data available regarding the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline) in humans, Linch et al. (1971) reported no effects in an occupational study involving 31 exposed and 31 nonexposed workers, but NIOSH (1986, 1987) located two cases of urinary bladder tumors in 370 workers in a 4,4'-methylene-bis(2-chloraniline) manufacturing plant.

Studies with laboratory animals associate bladder tumors in dogs (Stula et al., 1977), hepatomas in mice (Russfield et al., 1975) and lung tumors in rats (Stula et al., 1975; Kommineni et al., 1979) with oral exposure to 4,4'-methylene-bis(2-chloroaniline). Subcutaneous administration of 4,4'-methylene-bis(2-chloroaniline) to rats resulted in increased incidences of primary lung carcinomas and liver cell carcinomas compared with controls (Steinhoff and Grundmann, 1971).

The above animal studies provide positive evidence for the carcinogenicity of 4,4'-methylene-bis(2-chloroaniline). 4,4'-Methylene-bis(2-chloroaniline) was placed in CAG Group B2: probable human carcinogen.

Using the data presented in Table 9-4 and Appendix B and the multistage model by Howe et al. (1986), a human F factor of 0.79 $(mg/kg/day)^{-1}$ was estimated, which corresponds to a Potency Group of 3. Potency Group 3 compounds in U.S. EPA Group B are assigned a "low" hazard ranking, which corresponds to an RQ of 100 for carcinogenicity.

TABLE 9-4

Derivation of Potency Factor (F) for 4,4'-Methylene-bis(2-chloroaniline)

References: Stula et al., 1975; Kommineni et al., 1979

Species/strain/sex: Charles River CD/male and female; Charles River Sprague-Dawley/male

Route/vehicle: oral/diet

Length of exposure (le) = 2 years; 18 months

Length of experiment (LE) = 2 years; 2 years

Lifespan of animal (L) = 2 years

Body weight = see Appendix B-2 and B-3

Tumor type: adenocarcinoma; adenocarcinoma and all lung neoplasms

Adjusted $1/ED_{10}$ (F factor): 0.79 $(mg/kg/day)^{-1}$

Exposure	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
see Appendix B	see Appendix B	see Appendix B

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Windholz, M., S. Budavari, R.F. Blumetti and E.S. Otterbein. 1983. The Merck Index. Merck and Co., Inc., Rahway, NJ. p. 5929.

Yoneyama, K. and F. Matsumura. 1984. Microbial metabolism 4,4'-methylene-bis(2-chloroaniline). Arch. Environ. Contam. Toxicol. 13: 501-507.

APPENDIX A

LITERATURE SEARCHED

This HEED is based on data identified by computerized literature searches of the following:

CHEMLINE
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
TOXLINE
TOXLIT
TOXLIT 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register
CAS ONLINE (Chemistry and Aquatic)
HSDB
SCISEARCH
Federal Research in Progress

These searches were conducted in April, 1989, and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1987. TLVs: Threshold Limit Values for Chemical Substances in the Work Environment adopted by ACGIH with Intended Changes for 1987-1988. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1984. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., Littleton, MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. IARC, WHO, Lyons, France.

Jaber, H.M., W.R. Mabey, A.T. Lieu, T.W. Chou and H.L. Johnson. 1984. Data acquisition for environmental transport and fate screening for compounds of interest to the Office of Solid Waste. EPA 600/6-84-010. NTIS PB84-243906. SRI International, Menlo Park, CA.

NTP (National Toxicology Program). 1987. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, I.N. 1984. Dangerous Properties of Industrial Materials, 6th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1987. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1986. Report on Status Report in the Special Review Program, Registration Standards Program and the Data Call in Programs. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

USITC (U.S. International Trade Commission). 1986. Synthetic Organic Chemicals. U.S. Production and Sales, 1985, USITC Publ. 1892, Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.

APPENDIX

Cancer Data Sheet for Derivation of q_1^* Using Global86

Reference: Russfield et al., 1975

Species/strain/sex: mouse/Charles River CD-1/female

Route/vehicle: oral/food

Length of exposure (le) = 18 months

Length of experiment (LE) = 24 months

Lifespan of animal (L) = 24 months

Body weight = 0.03 (assumed)[a]

Tumor site and type: liver hepatomas

Purity: 97%

Exposure (ppm)	Transformed Dose[b] (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0/20
1000	97.5	9/21
2000	195	7/14

[a]Reference value from U.S. EPA (1980)

[b]Estimated by applying a reference food intake factor for mice of 0.13 kg diet/kg bw/day (US. EPA, 1980) and multiplying the result by 18/24 months to expand to continuous exposure

Unadjusted $q_1^* = 6.72965 \times 10^{-3}$ $(mg/kg/day)^{-1}$

Human $q_1^* = 8.9 \times 10^{-2}$ $(mg/kg/day)^{-1}$

Polynomial degree selection procedure and test selected by program using Monte Carlo test.

APPENDIX B-2

Cancer Data Sheet for Derivation of a q_1^* Using Global 86

Reference: Stula et al., 1975

Species/strain/sex: Rat/Charles River CD-1/male and female[a]

Route/vehicle: oral/food

Length of exposure (le) = 24 months

Length of experiment (LE) = 24 months

Lifespan of animal (L) = 24 months

Body weight = 0.35 (assumed)[b]

Tumor site and type: lung adenocarcinoma

Purity: 95%

Exposure (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0/88
1000	50	48/88

[a]Data from males and females were combined because neither gender appeared to be more sensitive.

[b]Reference value from U.S. EPA (1980)

[c]Estimated by applying a reference food intake factor of 0.05 kg diet/kg bw/day (U.S. EPA, 1980)

Unadjusted $q_1^* = 1.99485 \times 10^{-2}$ $(mg/kg/day)^{-1}$

Human $q_1^* = 1.2 \times 10^{-1}$ $(mg/kg/day)^{-1}$

Polynomial degree selection procedure and test selected by programming using Monte Carlo test.

Cancer Data Sheet for Derivation of a q_1^* Using Global 86

Reference: Kommineni et al., 1979

Species/strain/sex: rat/Charles River Sprague-Dawley/male

Route/vehicle: oral/food

Length of exposure (le) = 18 months

Length of experiment (LE) = 24 months

Lifespan of animal (L) = 24 months

Body weight = 0.59 for 0, 250 and 500 ppm groups; 0.52 for 1000 ppm group[a]

Tumor site and type: lung, all primary neoplasms

Purity: NR

Exposure	Transformed Dose[b] (mg/kg/day)	Equivalent Human Dose[c] (mg/kg/day)	Incidence No. Responding/No. Tested
0	0	0	1/100
250	9.4	1.91	23/100
500	18.8	3.83	28/75
1000	37.5	7.32	35/50

[a]Estimated from graphic data provided by investigators

[b]Estimated by applying a reference food intake factor of 0.05 kg diet/kg body weight (U.S. EPA, 1980) and multiplying the result by 18/24 months to expand to continuous exposure

[c]Estimated by muliplying the transformed animal dose by the cube root of the ratio of the rat body weight/human reference body weight of 70 kg

Human $q_1^* = 1.5 \times 10^{-1}$ $(mg/kg/day)^{-1}$

Polynomial degree selection procedure and test selected by program using Monte Carlo test.

NR = Not reported

APPENDIX C

Summary Table for MOCA

	Species	Exposure	Effect	RfD or q_1^*	Reference
Inhalation Exposure					
Subchronic	ID	ID	ID	ID	ID
Chronic	ID	ID	ID	ID	ID
Carcinogenicity	rat	oral, diet see Appendix B	lung tumors	1.3×10^{-1} (mg/kg/day)$^{-1}$	Stula et al., 1975 Komminent et al., 1979
Oral Exposure					
Subchronic	dog	100 mg/day, 3 days/week for 3 weeks, then 5 days/week for 9 years (7.3 mg/kg/day); LOAEL	liver injury, bladder inflammation	0.0007 mg/kg/day	Stula et al., 1977
Chronic	dog	100 mg/day, 3 days/week for 3 weeks, then 5 days/week for 9 years (7.3 mg/kg/day); LOAEL	liver injury, bladder inflammation	0.0007 mg/kg/day	Stula et al., 1977
Carcinogenicity	rat	oral, diet see Appendix B	lung tumors	1.3×10^{-1} (mg/kg/day)$^{-1}$	Stula et al., 1975 Komminent et al., 1979
REPORTABLE QUANTITIES					
Based on chronic toxicity:		1000			Komminent et al., 1979
Based on carcinogenicity:		100			Stula et al., 1975 Komminent et al., 1979

ID = Insufficient data

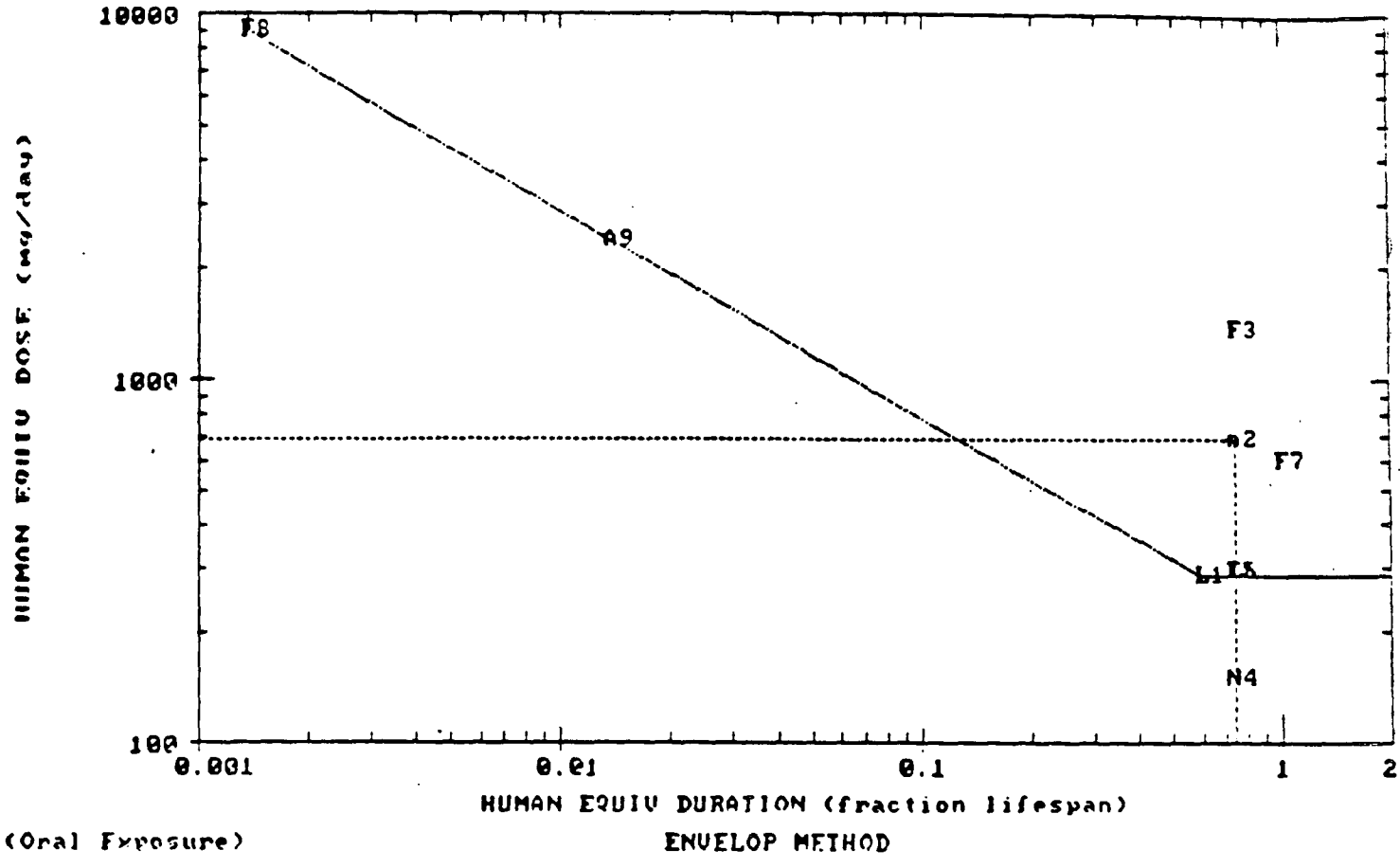
APPENDIX D

DOSE/DURATION RESPONSE GRAPHS FOR EXPOSURE TO 4,4-METHYLENE-BIS-(2-CHLOROANILINE)

D.1. DISCUSSION

Dose/duration-response graphs for oral exposure to 4,4'-methylene-bis-(2-chloroaniline) generated by the method of Crockett et al. (1985) using the computer software by Durkin and Meylan (1988) developed under contract to ECAO-Cincinnati are presented in Figures D-1 and D-2. Data used to generate these graphs are presented in Figure D-2. In the generation of these figures, all responses are classified as adverse (FEL, AEL or LOAEL) or nonadverse (NOEL or NOAEL) for plotting. For oral exposure, the ordinate expresses dosage as human equivalent dose. The animal dosage in mg/kg/day is multiplied by the cube root of the ratio of the animal:human body weight to adjust for species differences in basal metabolic rate (Mantel and Schneiderman, 1975). The result is then multiplied by 70 kg, the reference human body weight, to express the human equivalent dose as mg/day for a 70 kg human.

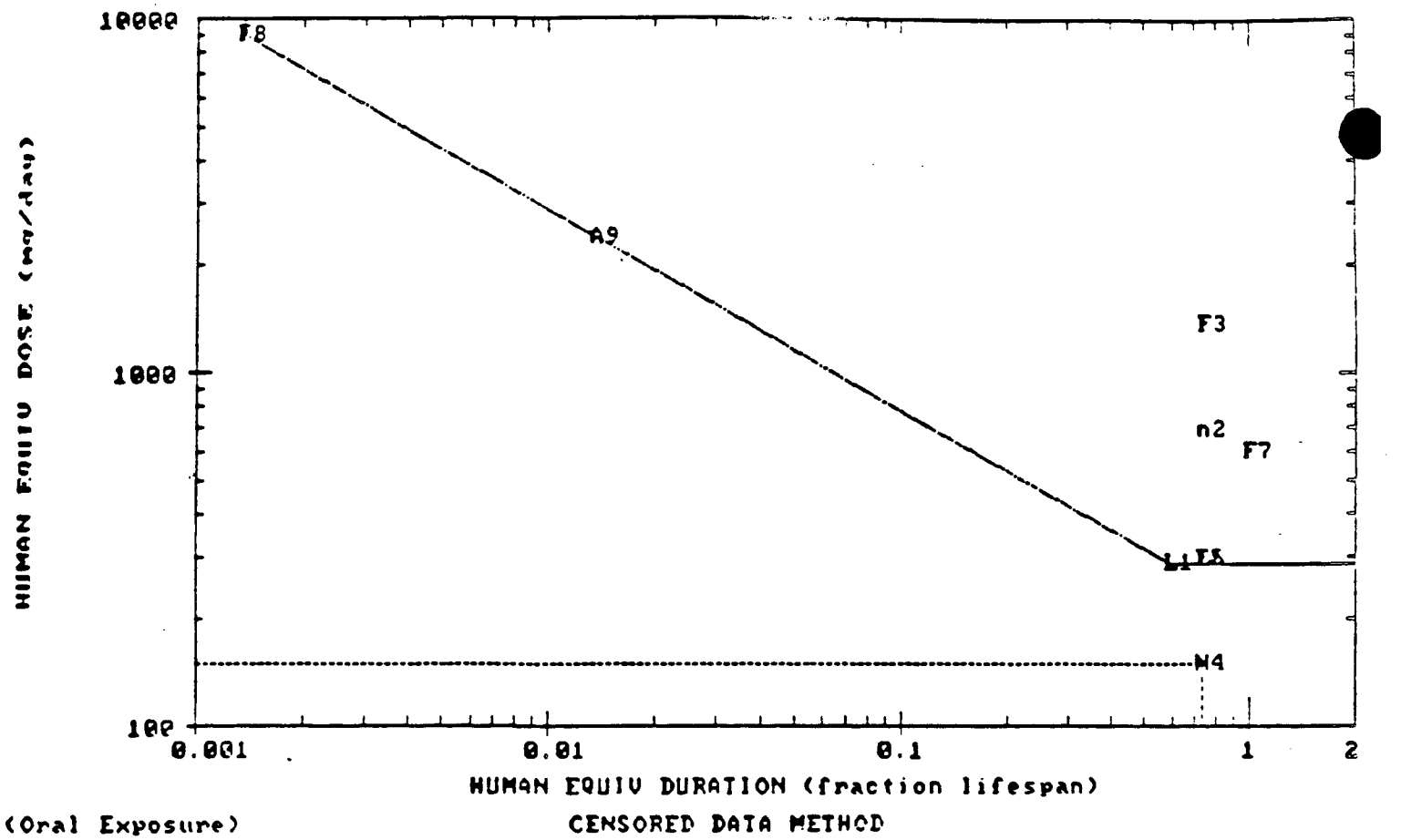
The boundary for adverse effects (solid line) is drawn by identifying the lowest adverse effect dose or concentration at the shortest duration of exposure at which an adverse effect occurred. From this point an infinite line is extended upward parallel to the dose axis. The starting point is then connected to the lowest adverse effect dose or concentration at the next longer duration of exposure that has an adverse effect dose or concentration equal to or lower than the previous one. This process is continued to the lowest adverse effect dose or concentration. From this point a line is extended to the right parallel to the duration axis. The region of adverse effects lies above the adverse effects boundary.



Key: F = FEL
L = LOAEL
A = AEL
N = NOEL
Solid line = Adverse Effect Boundary
Dashed line = No Adverse Effects Boundary

FIGURE D-1

Dose/Duration Response Graph for Oral Exposure to 4,4'-methylene-bis (2-Chloroaniline); Envelope Method



Key: F = FEL
L = LOAEL
A = AEL
N = NOEL
Solid line = Adverse Effect Boundary
Dashed line = No Adverse Effects Boundary

FIGURE D-2

Dose/Duration Response Graph for Oral Exposure to 4,4'-methylene-bis-(2-chloroaniline); Censored Data Method

Using the envelope method, the boundary for no adverse effects (dashed line) is drawn by identifying the highest no adverse effects dose or concentration. From this point, a line parallel to the duration axis is extended to the dose or concentration axis. The starting point is then connected to the next lower or equal no adverse effect dose or concentration at a longer duration of exposure. When this process can no longer be continued, a line is dropped parallel to the dose or concentration axis to the duration axis. The no adverse effects region lies below the no adverse effects boundary. At both ends of the graph between the adverse effects and no adverse effects boundaries are regions of ambiguity. The area (if any) resulting from intersection of the adverse effects and no adverse effects boundaries is defined as the region of contradiction.

In the censored data method, all no adverse effect points located in the region of contradiction are dropped from consideration and the no adverse effect boundary is redrawn so that it does not intersect the adverse effects boundary and no region of contradiction is generated. This method results in the most conservative definition of the no adverse effects region.

Figure D-1 presents the dose/duration-response graph for oral exposure generated by the envelope method. The adverse effects boundary is defined by an LD_{50} value in male rats (Rec. #8), a 10-day AEL in rats (Rec. #9) and the LOAEL for liver and urinary bladder effects in dogs (Rec. #1) that served as the basis of the RfD value for oral exposure. The only nonadverse effect levels are a NOAEL in mice (Rec. #2) and a NOEL in rats (Rec. #4) from studies that allowed a 6-month recovery period after exposure was terminated, which seriously erodes confidence in the designation of these two data points as nonadverse effect levels. The graph generated by the censored data method is presented in Figure D-2.

D.2. DATA USED TO GENERATE DOSE/DURATION-RESPONSE GRAPHS

D.2.1. Inhalation Exposure. Inhalation data suitable for graphing were not located.

D.2.2. Oral Exposure.

Chemical Name: 4,4'-Methylene-bis(2-chloroaniline) (MOCA)
CAS Number: 101-14-4
Document Title: Health and Environmental Effects Document for 4,4'-Methylene bis(2-Chloroaniline)
Document Number: Pending
Document Date: Pending
Document Type: HEED

RECORD #1:	Species:	Dogs		Dose:	7.300
	Sex:	Female		Duration Exposure:	9.0 years
	Effect:	LOAEL		Duration Observation:	9.0 years
	Route:	Capsul			
	Number Exposed:		5	5	
	Number Responses:		3	5	
	Type of Effect:		DEGEN	OTHER	
	Site of Effect:		LIVER	OTHER	
	Severity Effect:		5	5	

Comment: 100 mg/day 3 days/week for 6 weeks, 5 days/week for 9 years/liver injury and urinary bladder inflammation.

Citation: Stula et al., 1977

RECORD #2:	Species:	Mice		Dose:	130.000
	Sex:	Both		Duration Exposure:	18.0 months
	Effect:	NOAEL		Duration Observation:	24.0 months
	Route:	Food			
	Number Exposed:		50		
	Number Responses:		NR		
	Type of Effect:		HISTO		
	Site of Effect:		BODY		
	Severity Effect:		1		

Comment: 1000 ppm (doses studied: 1000, 2000 ppm); reduced amyloidosis.

Citation: Russfield et al., 1975

RECORD #3:

Species:	Mice	Dose:	260.000
Sex:	Female	Duration Exposure:	18.0 months
Effect:	FEL	Duration Observation:	24.0 months
Route:	Food		

Number Exposed:	25
Number Responses:	9
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: 2000 ppm (see previous record), reduced survival in females.

Citation: Russfield et al., 1975

RECORD #4:

Species:	Rats	Dose:	12.500
Sex:	Male	Duration Exposure:	540.0 days
Effect:	NOEL	Duration Observation:	720.0 days
Route:	Food		

Number Exposed:	100
Number Responses:	0
Type of Effect:	
Site of Effect:	
Severity Effect:	3

Comment: 250 ppm (Doses studied: 250, 500, 1000 ppm).

Citation: Kommineni et al., 1979

RECORD #5:

Species:	Rats	Dose:	25.000
Sex:	Male	Duration Exposure:	540.0 days
Effect:	FEL	Duration Observation:	720.0 days
Route:	Food		

Number Exposed:	75
Number Responses:	65
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: 500 ppm (see previous record); rats at 1000 ppm also had reduced rate of body weight gain.

Citation: Kommineni et al., 1979

RECORD #6:

Species:	Rats	Dose:	25.000
Sex:	Male	Duration Exposure:	18.0 months
Effect:	LOAEL	Duration Observation:	24.0 months
Route:	Food		

Number Exposed:	25
Number Responses:	NR
Type of Effect:	WGTDC
Site of Effect:	BODY
Severity Effect:	4

Comment: 500 ppm (Doses studied: 500, 1000 ppm); dose-related decrease in terminal body weights.

Citation: Russfield et al., 1975

RECORD #7:

Species:	Rats	Dose:	50.000
Sex:	Both	Duration Exposure:	2.0 years
Effect:	FEL	Duration Observation:	2.0 years
Route:	Food		

Number Exposed:	100	100
Number Responses:	NR	NR
Type of Effect:	DEATH	NECRO
Site of Effect:	BODY	LIVER
Severity Effect:	10	6

Comment: 1000 ppm; other liver effects: hepatocytomegaly, fatty change, bile duct proliferation and fibrosis

Citation: Stula et al., 1975

RECORD #8:

Species:	Rats	Dose:	750.000
Sex:	Male	Duration Exposure:	1.0 days
Effect:	FEL	Duration Observation:	1.0 days
Route:	Oral (NOS)		

Number Exposed:	NR
Number Responses:	NR
Type of Effect:	DEATH
Site of Effect:	BODY
Severity Effect:	10

Comment: Lowest LD_{50} reported in rats.

Citation: Miller and Sherman, 1965

RECORD #9:	Species:	Rats		Dose:	200.000
	Sex:	NR		Duration Exposure:	10.0 days
	Effect:	AEL		Duration Observation:	10.0 days
	Route:	Oral (NOS)			
	Number Exposed:	NR	NR	NR	
	Number Responses:	NR	NR	NR	
	Type of Effect:	WGTDC	HEMAT	EXCRE	
	Site of Effect:	BODY	BLOOD	N.S.	
	Severity Effect:	4	UNCL	UNCL	

Comment: Cyanosis, pallor, "blood and urine abnormalities."

Citation: Reinke, 1963

NR = Not reported