

2-CHLORO-1,4-BENZENEDIAMINE

CAS # 615-66-7

ORAL RISK ASSESSMENT DOCUMENT



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EXECUTIVE SUMMARY

2-CHLORO-1,4-BENZENEDIAMINE – Oral Risk Assessment CAS # 615-66-7			
PARAMETER	LEVEL	UNITS	DERIVED
BMDL₁₀ (95% confidence limit at 10% response level)	15	mg/kg-day	From a chronic feeding study in rats
Oral RfD (oral reference dose)	0.05	mg/kg-day	From the BMDL ₁₀ with a 300x total uncertainty factor
TAC (total allowable concentration)	0.3	mg/L	For a 70 kg adult drinking 2 L/day, with a 20% relative source contribution for drinking water
SPAC (single product allowable concentration)	0.03	mg/L	From the TAC, assuming 10 potential sources of 2-chloro-1,4-benzenediamine in drinking water
STEL (short term exposure level)	0.5	mg/L	From a chronic rat feeding study and based on a 10 kg child drinking 1 L/day.
KEY STUDY	NTP/NCI (National Toxicology Program/National Cancer Institute). 1978a. Bioassay of 2-Chlorophenylenediamine Sulfate for Possible Carcinogenicity. Technical Report Series No. 113 DHEW Publication No. (NIH) 78-1368, U.S. Department of Health Education and Welfare, National Cancer Institute, Bethesda, MD 20014.		
CRITICAL EFFECT	Transitional cell hyperplasia of the kidney and renal pelvis in male rats.		
UNCERTAINTY FACTORS	Uncertainty factors applied in calculating the oral RfD are as follows: • 10x for interspecies extrapolation • 10x for intraspecies extrapolation • 1x for extrapolation from a less-than-lifetime study to lifetime duration • 1x for extrapolation from a LOAEL to a NOAEL • 3x for database deficiencies. The total uncertainty factor is, therefore, 300x.		
TOXICITY SUMMARY	No oral data in humans were available. 2-Chloro-1,4-benzenediamine sulfate did not cause a statistical increase in any tumor type in rats or mice after chronic dietary administration. However, transitional cell hyperplasia of the kidney and renal pelvis was observed in male and female rats at increased incidence compared to controls. The incidence of renal epithelial hyperplasia was dose related in males, but a NOAEL could not be identified. Hepatic focal necrosis was observed in male mice at an increased incidence compared to controls, but the incidence was not dose-related. No kinetic and limited metabolism studies in humans and laboratory animals were identified for 2-chloro-1,4-benzenediamine. In the <i>Salmonella typhimurium</i> reverse mutation assay, 2-chloro-1,4-benzenediamine sulfate produced dose-related increases in revertant colonies of more than twice the background level at higher doses but in the absence of cytotoxicity. 2-Chloro-1,4-benzenediamine was negative in the <i>in vivo</i> alkaline single cell assay (Comet assay) and in the alkaline elution assay for the detection of hepatic DNA damage. The limited genotoxicity data identified for 2-chloro-1,4-benzenediamine or its sulfate salt precluded definitive conclusions regarding its genotoxic potential. However, structure-activity relationship studies suggest that the genotoxic or carcinogenic potential of 2-chloro-1,4-benzenediamine is less than that of 4-chloro-1,2-benzenediamine or 4-chloro-1,3-benzenediamine. Further, no statistical increases in tumors were observed after chronic feeding. The <i>data are inadequate for an assessment of human carcinogenic potential</i> of 2-chloro-1,4-benzenediamine or its sulfate salt. For the purposes of this risk assessment, 2-chloro-1,4-benzenediamine was considered a non-carcinogen. A NOAEL could not be identified for the critical effect of transitional cell hyperplasia of the kidney and renal pelvis in male rats. A BMDL₁₀ of 15 mg/kg-day was determined, since the incidence was dose-related. This effect was considered non-neoplastic, since the rats were treated for at least 24 months, as recommended by current U.S. EPA health effects testing guidelines, and the effect did not progress into renal tumors.		
CONCLUSIONS	The drinking water action levels developed in this risk assessment are protective of public health since they were developed based on chronic oral data for 2-chloro-1,4-benzenediamine sulfate from the most sensitive endpoint and laboratory animal species.		

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **2-chloro-1,4-benzenediamine** in drinking water, as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2004), or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2004). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993; U.S. EPA, 2002), which assumes that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996; U.S. EPA, 2002) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED₁₀ or BMDL₁₀ from benchmark dose programs) can be used (U.S. EPA, 2003a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime” (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 2003b).

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

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

or

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

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

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

Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED₁₀ of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to a level at or below the TAC under static conditions, or to a level at or below the SPAC under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a, U.S. EPA, 1999; U.S. EPA, 2003c; U.S. EPA, 2005). If there is sufficient evidence to use a non-linear model, the LED₁₀ or BMDL₁₀, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED₁₀ or BMDL₁₀ to zero, is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10⁻⁶ to 10⁻⁴ is considered by the U.S. EPA to be safe and protective of public health (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2004) and 61 (2004), the TAC is set at the 10⁻⁵ risk level, and the SPAC is set at the 10⁻⁶ risk level. Use of a higher risk level is not ruled out, but would generally require documentation of a benefit to counteract the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2004) and 61 (2004). Application of these values to other exposure scenarios should be done with care, and with a full understanding of the derivation of the values and of the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation, and require the use of additional uncertainty factors to protect public health.

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

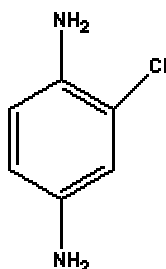
The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

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

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

2.0 PHYSICAL AND CHEMICAL PROPERTIES

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



2-Chloro-1,4-benzenediamine has synonyms of 2-chloro-4-phenylenediamine, 2-chloro-p-phenylenediamine, 3-chloro-4-aminoaniline, and 1,4-diamino-2-chlorobenzene (ChemIDPlus, 2004; HSDB, 2004). It has a trade name of Ursol Brown O (HSDB, 2004).

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

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

Property	Data	Reference
Empirical Formula	C ₆ H ₇ ClN ₂	HSDB, 2004
CAS#	615-66-7	HSDB, 2004
Molecular Weight	142.6	HSDB, 2004
Physical State and Color	Not located	
Melting Point	64 °C 250-262 °C (sulfate salt)	CIR, 1992 NTP/NCI, 1978a
Boiling Point	Not located	
Density	Not located	
Vapor Pressure	Not located	
Water Solubility	Not located	
pK _a	Not located	
n-Octanol/Water Partition Coefficient (log K _{ow})	0.25 (estimated)	SRC, 2004
Henry's Law Constant (air/water partition)	Not located	

2.1 Organoleptic Properties

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

3.0 PRODUCTION AND USE

3.1 Production

2-Chloro-1,4-benzenediamine is not produced commercially in the United States (HSDB, 2004). No other information regarding the production of 2-chloro-1,4-benzenediamine or its sulfate salt, including a method of production, were located.

3.2 Use

2-Chloro-1,4-benzenediamine is used as an intermediate in dyestuffs and pigments (Sontag, 1981) and as a rubber-processing agent (IARC, 1982). It is also a component of commercial hair dyes, and 2-chloro-1,4-benzenediamine or the corresponding sulfates are primary intermediates in at least 31 commercially available permanent hair dyes (NTP, 2004). Data submitted to the FDA by cosmetics firms in 1984 indicated that 2-chloro-1,4-benzenediamine was used in one formulation at a concentration of up to 1% (CIR, 1992). 2-Chloro-1,4-benzenediamine sulfate was reportedly used in 61 hair dyes and colors, 25 of which were at concentrations of 0.1-1%, and 36 of which were at concentrations less than 0.1% (CIR, 1992). However, no current references to its use in the United States as a dye were located, and IARC (1982) reported that no evidence was found that its isomers 4-chloro-1,3-benzenediamine or 4-chloro-1,2-benzenediamine are presently employed commercially in the United States as a dye or dye intermediate.

No sanctioned uses for 2-chloro-1,4-benzenediamine as a direct or indirect food additive were identified under Title 21 of the U.S. Code of Federal Regulations (U.S. FDA, 2004). However, a number of non-halogenated phenylenediamine derivatives used as antioxidants and antiozonants in rubber articles intended for repeated food contact are listed in 21 CFR section 177.2600.

These derivatives are frequently used in products evaluated by NSF, such as gaskets, O-rings, and other generally small rubber parts with the potential to contact drinking water.

4.0 ANALYTICAL METHODS

4.1 Analysis in Water

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

The analysis of 2-chloro-1,4-benzenediamine in aqueous environmental samples using high performance liquid chromatography with either an ultraviolet or an electrochemical detector has been described by Riggin and Howard (1983). The water samples were extracted with methylene chloride. The electrochemical method at 0.7 Volts had a better detection limit compared to the ultraviolet method at 254 nanometers. Using electrochemical detection, the estimated detection limit for 2-chloro-1,4-benzenediamine was 0.2 ng of injected chemical.

4.2 Analysis in Biological Matrices

No information pertaining to the analysis of chlorobenzenediamine isomers in biological matrices was located.

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

No information on the extent of human exposure to 2-chloro-1,4-benzenediamine was located. Exposure may occur occupationally or through the manufacture or use of certain dyes or pigments, since 2-chloro-1,4-benzenediamine is reportedly used as an intermediate in dyestuffs and pigments (Sontag, 1981). No further information was located. 2-Chloro-1,4-benzenediamine is occasionally detected at concentrations up to 100 µg/L in laboratory extractant water samples of products evaluated by NSF. 2-Chloro-1,4-benzenediamine is not used directly in material formulations. However, benzenediamine compounds are used as ingredients in membranes and as rubber additives, and may react with available chlorine from other ingredients in a material formulation or in drinking water.

5.2 Sources of Environmental Exposure

No information was located regarding the potential for environmental exposure to 2-chloro-1,4-benzenediamine. Due to its industrial uses, environmental exposure could occur if accidental releases of the compound into the environment took place.

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

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

7.0 EFFECTS ON HUMANS

7.1 Case Reports

Skin irritation, asthmatic symptoms, and eye damage have been reported following exposure to 2-chloro-1,4-benzenediamine sulfate, but no further details were available (NTP/NCI, 1978a). Hansson and Thorneby-Andersson (2001) reported two cases of women with eczematous reactions on the lashes and brows after using semi-permanent cream dyes. Both women displayed strong reactions to patch testing with 2-chloro-1,4-benzenediamine in 1% petrolatum when read on Day 3. Both women also cross-reacted to patch testing of other phenylenediamine derivatives.

7.2 Epidemiological Studies

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

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

Although 2-chloro-1,4-benzenediamine did not cause primary dermal irritation in the rabbit, it was reported to be a strong dermal sensitizer in guinea pigs. Mild conjunctival inflammation was observed in rabbits in an assay for primary ocular irritation using 2-chloro-1,4-benzenediamine.

Subchronic (eight-week) administration of 2-chloro-1,4-benzenediamine sulfate was associated with a reduction in mean body weight in female mice, but male mice and rats were not affected (decreases of < 10%). Although a 13-week feeding study was also reported in the literature for 2-chloro-1,4-benzenediamine sulfate, limited details were available.

Transitional cell hyperplasia of the kidney and renal pelvis was observed in rats at an increased incidence compared to controls after chronic dietary administration of 2-chloro-1,4-benzenediamine sulfate, and the incidence was dose related in males. Hepatic focal necrosis was observed in male mice at an increased incidence compared to controls after chronic dietary administration of 2-chloro-1,4-benzenediamine sulfate, but the incidence was not dose-related. 2-Chloro-1,4-benzenediamine sulfate did not cause a statistically significant increase in any tumor type in rats or mice after chronic dietary administration. 2-Chloro-1,4-benzenediamine did not produce an increase in gross, visceral, or skeletal anomalies at a dose producing significant maternal, embryo, and fetal toxicity. Thus, 2-chloro-1,4-benzenediamine was not a developmental toxicant in rats in the absence of maternal toxicity.

In the *Salmonella typhimurium* reverse mutation assay, 2-chloro-1,4-benzenediamine sulfate produced dose-related increases in revertant colonies of more than twice the background level in the absence of cytotoxicity. 2-Chloro-1,4-benzenediamine was negative in the *in vivo* alkaline single cell assay (Comet assay) and in the alkaline elution assay for the detection of hepatic DNA damage. No other genotoxicity data, including chromosomal aberration data, were identified.

8.1 Limited-Exposure Effects

8.1.1 Irritation and Sensitization Studies

2-Chloro-1,4-benzenediamine was evaluated in a primary dermal irritation assay (CIR, 1992). Patches containing 0.5 mL of a 2.5% (w/v) solution of 2-chloro-1,4-benzenediamine (unspecified purity in distilled water) were applied to two unspecified sites, one intact and one abraded, of clipped skin on three albino rabbits. After a 24-hour exposure, the patches were removed, and the test sites were graded on a scale of 0 to 4. The test sites were also graded 72 hours after exposure. According to the authors, no reactions were observed, but the data were not provided.

2-Chloro-1,4-benzenediamine was evaluated in the Magnusson and Kligman sensitization assay in guinea pigs (CIR, 1992). Fifteen female Pirbright white guinea pigs each received five intracutaneous injections (unspecified volume) of 0.3% 2-chloro-1,4-benzenediamine (unspecified purity in distilled water) over a five-day period. Ten female Pirbright white guinea pigs served as controls. After a four-week non-treatment period, the guinea pigs were patch-tested with 0.003, 0.006, 0.03, and 0.3% 2-chloro-1,4-benzenediamine. Reactions were scored after 24 and 48 hours. Although it was reported that 2-chloro-1,4-benzenediamine was strongly sensitizing, no data were provided, and no further details were available.

8.1.2 Ocular Exposure Studies

In a test for primary ocular irritation, a 2.5% (w/v) solution of 2-chloro-1,4-benzenediamine (unspecified purity in sodium sulfite-buffered water) was instilled into one eye of each of three rabbits (CIR, 1992). Although not stated, it was assumed that the untreated eye served as the negative control. Ten seconds after instillation, the eyes were rinsed with 20 mL of distilled water. One rabbit exhibited mild conjunctival inflammation, but no reactions were observed in the other two rabbits.

8.2 Single-Exposure Studies

No LD₅₀ studies were identified for 2-chloro-1,4-benzenediamine or 2-chloro-1,4-benzenediamine sulfate.

8.3 Short-Term Exposure Studies

Female Sprague-Dawley rats were administered 2-chloro-1,4-benzenediamine (purity unspecified in water vehicle) at 0, 63, or 189 mg/kg via gavage (Kitchin et al., 1993). The low dose was 2.6 times the maximum tolerated dose from the NTP/NCI (1978a) cancer bioassay. The control and treated groups contained seven and nine rats, respectively. Food and water were

available *ad libitum*. A second dose was administered 17 hours after the first dose, and the rats were sacrificed four hours later, after a total of 21 hours. Hepatic cytochrome P450 content and hepatic ornithine decarboxylase activity were measured as markers for promotion of carcinogenesis. Serum alanine aminotransferase activity was measured as a marker for cell toxicity. The 4- and 21-hour time points were selected because according to the study authors, hepatic ornithine decarboxylase activity responds well to a 4-hour chemical pretreatment, while hepatic cytochrome P450 content and serum alanine aminotransferase activity were better measured after 21 hours. The purpose of this study was to determine whether the results of these simple biochemical assays could be used to predict the outcome of rodent carcinogenicity assays.

No rats died. At 63 mg/kg, mean hepatic ornithine decarboxylase activity was statistically increased by approximately 85% compared to controls. At 189 mg/kg, mean hepatic cytochrome P450 content was statistically increased by approximately 40% compared to controls. Mean serum alanine aminotransferase activity was not statistically different from controls at either dose level. The study authors did not provide an explanation with respect to the lack of a dose-response for the change in ornithine decarboxylase activity or hepatic cytochrome P450 content.

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

8.4 Long-Term and Chronic Exposure Studies

8.4.1 Subchronic Studies

Five Fischer 344 rats per sex and dose were administered 2-chloro-1,4-benzenediamine sulfate (purity unspecified) at dietary levels of 0, 0.03, 0.1, 0.3, 1.0, or 3.0% for eight weeks (NTP/NCI, 1978a; CIR, 1992). These doses were equivalent to 2-chloro-1,4-benzenediamine doses of 0, 18, 59, 178, 590, or 1,780 mg/kg-day in males and 0, 20, 67, 201, 670, or 2,010 mg/kg-day in females, respectively (see Appendix 13.1). Individual body weights and daily food consumption per cage were recorded throughout the study. At study termination, all survivors were sacrificed and necropsied. At 0.3%, no rats died, but mean body weight was depressed 10.4% in males and 6.4% in females. At 1%, all male rats and one female rat died. The results for rats administered the highest dose were not reported. No further details were provided. This study was conducted to determine dose levels for a chronic feeding study and, as such, did not examine all the endpoints required under current regulatory guidelines (U.S. EPA, 2003d).

Five B6C3F1 mice per sex and dose were administered 2-chloro-1,4-benzenediamine sulfate (purity unspecified) at dietary levels of 0, 0.03, 0.1, 0.3, 1.0, or 3.0% for eight weeks (NTP/NCI, 1978a; CIR, 1992). These doses were equivalent to 2-chloro-1,4-benzenediamine doses of 0, 32, 107, 320, 1,070, or 3,200 mg/kg-day in males and 0, 35, 116, 350, 1,160, or 3,500 mg/kg-day in females, respectively (see Appendix 13.1). Individual body weights and daily food consumption per cage were recorded throughout the study. At study termination, all survivors were sacrificed and necropsied. At 0.3%, no mice died, and mean body weight was depressed 16.1% in females. At 1%, one of the males died, and mean body weight was depressed 3.8% in males and 14.4% in females. The results for mice administered the highest dose were not reported. No further details were provided. This study was conducted to determine dose levels for a chronic feeding study.