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Provisional Peer Reviewed Toxicity Values for

N-Nitrosodimethylamine
(CASRN 62-75-9)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR N-NITROSODIMETHYLAMINE (CASRN 62-75-9)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

IRIS (U.S. EPA, 2007) did not list an oral RfD for N-nitrosodimethylamine. An RfD for N-nitrosodimethylamine was not listed in the HEAST (U.S. EPA, 1997) or the Drinking Water and Health Advisories List (U.S. EPA, 2004). The CARA database (U.S. EPA, 1991, 1994) listed a Health and Environmental Effects Profile (HEEP) for nitrosamines (U.S. EPA, 1986) that did not derive an RfD for N-nitrosodimethylamine because of its carcinogenicity in chronic animal studies. A Toxicological Profile on N-nitrosodimethylamine (ATSDR, 1989) did not derive oral MRLs. ATSDR concluded that MRL derivation was precluded because there was not enough information to characterize the threshold region (NOAELs) for intermediate and chronic exposure, and noted that perinatal mortality occurred at a lower dose than available NOAELs for liver effects.

IRIS (U.S. EPA, 2007) did not list an inhalation RfC for N-nitrosodimethylamine, nor was an RfC listed in the HEAST (U.S. EPA, 1997). The HEEP for nitrosamines (U.S. EPA, 1986) contained no information regarding long-term inhalation toxicity of N-nitrosodimethylamine and, therefore, did not derive an RfC. ATSDR (1989) did not derive inhalation MRLs for N-nitrosodimethylamine due to the lack of appropriate data. The

toxicological profile included reports of impaired blood clotting and hepatic toxicity (necrosis, cirrhosis) in humans and animals following acute inhalation exposures. ATSDR (1989) also reported cancers of the nose, liver, kidney, and lung, but no noncancer effects in animals following chronic inhalation exposure to N-nitrosodimethylamine.

Both a cancer classification and an oral slope factor for N-nitrosodimethylamine were available on IRIS (U.S. EPA, 2007). The cancer assessment, last revised in 1993, classified N-nitrosodimethylamine in category B2 (probable human carcinogen under 1986 Guidelines for Carcinogen Assessment) based on tumors at multiple sites in both rodent and nonrodent mammals exposed by various routes. IRIS (U.S. EPA, 2007) reported an oral slope factor of 51 per mg/kg-day based on liver tumors in female Colworth rats exposed via drinking water (Peto et al., 1984). IRIS also reported an inhalation unit risk of 0.014 per $\mu g/m^3$ for N-nitrosodimethylamine based on extrapolation from the oral data (Peto et al., 1984). The present document does not include a cancer assessment for N-nitrosodimethylamine, because one was available on IRIS.

ACGIH (2005) did not list a quantitative TLV for N-nitrosodimethylamine, but assigned it a skin notation, for the possibility of cutaneous absorption, and an A3 notation as a confirmed liver carcinogen in animals with unknown relevance to humans. Under the A3 category, occupational exposures to N-nitrosodimethylamine are to be kept as low as possible. Similarly, NIOSH (2006) did not list a REL for N-nitrosodimethylamine since it a proven carcinogen and exposures are to be minimized. OSHA (2006) did not list a PEL for N-nitrosodimethylamine, but listed it as a Regulated Cancer Suspect Agent. The NTP (2006) Management Status Report indicated that a toxicogenomic rat liver evaluation of N-nitrosodimethylamine administered in drinking water was underway (study commenced in February, 2006). Information in the database indicated that the study will expose rats to either 0 or 5 ppm in drinking water for 8 or 13 weeks. This study will include a complete histopathological examination and may provide data on nonneoplastic endpoints.

N-nitrosodimethylamine also is known widely as dimethylnitrosamine. Other sources of information included a Concise International Chemical Assessment Document (CICAD; WHO, 2002), an Environmental Health Criteria document on nitroso compounds (WHO, 1978), an IARC (1978) monograph on N-nitrosodimethylamine, and a chapter on nitroso compounds in Patty's Industrial Hygiene and Toxicology (Lijinsky, 2001). In general, these review documents characterized N-nitrosodimethylamine as a potent animal carcinogen in all species tested and indicated that chronic oral exposures induced tumors of liver, lung, and kidney. Literature searches from 1985 to November 2000 were conducted on TOXLINE, MEDLINE, and TSCATS to obtain information relevant to the derivation of an RfD or RfC for N-nitrosodimethylamine. Update literature searches of MedLine, ToxLine, ToxCenter, TSCATS, CCRIS, DART/ETIC, GENETOX, RTECS, HSDB, and Current Contents were conducted in January, 2006.

REVIEW OF PERTINENT DATA

Human Studies

Several review documents reported hepatic toxicity in humans following acute oral or inhalation exposure to N-nitrosodimethylamine, but no data on subchronic or chronic exposures (U.S. EPA, 1986; ATSDR, 1989; Lijinsky, 2001). No other relevant information was identified in the literature searches.

Animal Studies

Preussman and Stewart (1984) provided a thorough summary of early data on the toxicity, carcinogenicity, and metabolism of this and other n-nitroso chemicals.

Oral Exposure

Subchronic Studies

Khanna and Puri (1966) fed groups of 25 albino rats (gender unspecified) a standard diet containing 0 or 75 ppm of N-nitrosodimethylamine for up to 12 weeks. Estimating from the initial body weight data and an allometric equation for rat food consumption (U.S. EPA, 1988), the intake of N-nitrosodimethylamine was calculated to be 0 or 6.8 mg/kg-day. After 1, 2, 4, 8, and 12 weeks, a few individuals in each group were sacrificed and several organs (liver, spleen, kidney, lung, and myocardium) were examined for histopathology. No quantitative data were supplied in this report. N-nitrosodimethylamine-treated rats had progressively reduced body weights and absolute liver weights compared to controls. The first signs of hepatic necrosis with hemorrhage appeared near the central veins after one week of treatment with N-nitrosodimethylamine and extended to the entire liver after four weeks. Regenerative nodules appeared in the liver at 12 weeks. None of these alterations were observed in control animals. Results from parallel experiments demonstrated that the progress of N-nitrosodimethylamine-generated liver disease was accelerated in rats receiving diets deficient in protein, "L-cystine", or choline. The 6.8 mg/kg-day (75 ppm) level represents a FEL because of severe hepatic effects (massive hemorrhagic necrosis, reduced absolute liver weight) and reduced body weight gain.

Barnes and Magee (1954) fed groups of 6 young albino rats (gender unspecified) a diet containing 0, 50, 100, or 200 ppm of N-nitrosodimethylamine in arachis oil for 110 days. Using a reference body weight and an allometric equation for food consumption (U.S. EPA, 1988), the intake of N-nitrosodimethylamine was calculated as 0, 5.9, 11.7, or 23.4 mg/kg-day. Treatment with N-nitrosodimethylamine at the two higher dose levels was fatal: all 200 ppm rats died in the fifth week of treatment and all 100 ppm rats died after 8-13 weeks of treatment. No mortality occurred in the 50 ppm group. Body weights of all treated rats were significantly lower than controls; in the two higher exposure groups, rats became emaciated, completely lacking body fat, and the mean weights of the 100 ppm group were reported to be reduced by one-third compared to controls. Information on the degree of body weight reduction in the 50 ppm group was not

provided in the study. Rats receiving 100 or 200 ppm exhibited hemorrhage of the gut and hepatic effects (abnormally small liver, varying degrees of necrosis, fibrosis in rats 'dying later'). The 5.9 mg/kg-day (50 ppm) exposure level was a LOAEL for reduced body weight. However, this study was not suitable for risk assessment because the results were not reported completely or quantitatively.

Jenkins et al. (1985) administered N-nitrosodimethylamine (1-5 μL/kg-day) by gavage to Wistar rats for 4 to 16 weeks with varying intervals between treatments in order to develop an animal model for cirrhosis of the liver. The treatment selected to optimize the cirrhosis effect was a daily dose of 2.5 μL/kg-day in two 4-week treatments, separated by an off-week. Little information was provided about the other treatment levels, except that higher doses resulted in increased mortality and lower doses failed to produce cirrhosis. Based on a density of 1.0061 g/mL, the intake of 2.5 μL/kg-day is equivalent to 2.5 mg/kg-day. At this level, mortality was approximately 42% two weeks after cessation of treatment and 50% by 20 weeks after treatment. Treatment-associated changes occurred in serum chemistry parameters: significant increases in alkaline phosphatase (ALP) activity, gamma-glutamyl transferase (GGT) activity, and total and direct bilirubin levels, and significant reductions in concentrations of total protein, globulin, and albumin. Most of these changes persisted 24 weeks after treatment ceased. Portal hypertension was significantly developed in treated rats and became progressively worse from 2 to 24 weeks after treatment. Concomitantly, blood flow through the liver significantly decreased. Histopathological changes in the liver produced by N-nitrosodimethylamine included focal nodularity and sporadic necrosis observed 2 weeks after the end of treatment and well-developed cirrhosis with diffuse nodularity and fibrosis by 24 weeks after the end of treatment. The 2.5 mg/kg-day treatment level was a FEL because of increased mortality.

In a cancer study, Takayama and Oota (1965) gave three strains of mice (gender unspecified) diets containing N-nitrosodimethylamine in peanut oil for 5 or 10 months and observed them for their remaining life span. Thirty C3H mice were fed 50 ppm for 5 months, for an average daily intake of 5.26 mg/kg-day; ten untreated controls were observed for 18 months. No control groups were reported for the other two strains. ddN mice (n= 27 or 20) received 50 or 100 ppm, for average daily intakes of 7.05 or 17.7 mg/kg-day, respectively. Groups of 20-22 ICR mice were fed 50 or 200 ppm for 5 months or 50 ppm for 10 months, for average daily intakes of 6.65, 18.9, or 9.04 mg/kg-day, respectively. Complete necropsies and histopathology were performed on all dead animals. Lifespan was shortened in animals treated with N-nitrosodimethylamine. The average time to death ranged from 7.5 to 11.0 months after the commencement of exposure in all treatment groups, compared with 18 months in control C3H mice. Most of the treated mice showed severe hepatic effects (coagulative necrosis in association with dilatation of sinusoids, occasional focal hyperplasia of stellate cells and patchy disappearance of hepatocytes in 50% of the animals). All treatment groups showed a significant increase in various carcinomas or adenomas of lung or liver compared to controls. The lowest treatment level, 5 mg/kg-day in C3H mice was a FEL because of lowered survival due to severe hepatic necrosis.

Koppang and Rimeslatten, (1976) fed five groups of twelve minks of each gender a standard diet supplemented with 10% fish meal for 122 days. Diets of treated minks included the doses of N-nitrosodimethylamine summarized in Table 1. All animals appeared well

Table 1. Doses of N-Nitrosodimethylamine in Mink Feed for 122 Days

Minks	NDMA mg/kg feed	NDMA mg/kg total	NDMA mg/kg-day	Pathoanatomical changes	Notes	Minks bred
12 males	2.4	5	0.04	No		3
12 females	2.4	7	0.06	No		2
12 males	3.5	8	0.06	No		0
12 females	3.5	10	0.08	No	NOAEL	3
12 males	2.2	5	0.04	No		1
12 females	2.2	6	0.05	No		4
12 males	7.2	16	0.13	Yes	LOAEL	2
12 females	7.2	21	0.17	Yes		5

*Koppang and Rimeslatten, 1976

nourished with no gross lesions. However, histological examinations revealed small obliterative changes in some branches of hepatic veins in the high dose animals. Sixteen mink of this generation died spontaneously following rupture of liver tumors.

Two to five females per dose group and one to three males from each of the lower dose groups were not examined, but were kept in the trial for breeding; all subsequently were fed at the highest dose. Of the 14 females mated, 11 produced a total of 44 kits. Only 30 kits survived until weaning at age 3 months. Of these, 23 survived to be fed the maximum dose diet for their remaining lives. Their growth rates were slightly below those of controls and none exhibited signs of disease. Of these, six were commercially skinned after 178 days, 14 died, and only the remaining three survived until the end of the study, at day 544. Mean F1 survival times were 271 days for females. Males, which were fed only 0.12 mg/kg-day, survived a mean 393 days. Two litters were obtained from this generation. The first litter of two kits died two days after delivery. Of the second litter of three kits, one survived to represent the F2 generation. This study revealed a subchronic NOAEL of 60 ug/kg-day in male minks and 80 ug/kg-day in females for hepatic vein occlusion. The LOAELs were 130 and 170 ug/kg-day, respectively.

Anderson et al. (1986) exposed groups of 10-20 female outbred Swiss Cr:NIH(s) mice to 0 or 50 ppm of N-nitrosodimethylamine in drinking water for up to 4 weeks. Based on the reported body weight and water consumption data, the intake of N-nitrosodimethylamine was calculated as 0 or 5 mg/kg-day. At termination, mice were necropsied and gross liver lesions were scored on a semiquantitative scale. Part of each liver was analyzed for N-nitrosodimethylamine *N*-demethylase activity and part was evaluated for histopathology, also scored semiquantitatively. Exposure to N-nitrosodimethylamine significantly reduced body weight (by ~11%) and reduced water consumption by ~50%. Treated mice exhibited gross and histological signs of hepatotoxicity after the first week of exposure. After four weeks of treatment, gross effects included pits, yellowing or darkening of the liver, and moderate

accumulation of peritoneal fluid. Histologically, the livers exhibited mild centrilobular hemorrhage. N-nitrosodimethylamine *N*-demethylase activity was not significantly altered during treatment. This study identified a LOAEL of 5 mg/kg-day for hepatic toxicity including centrilobular hemorrhage.

Boothe et al. (1992) administered initial doses of 0 or 2 mg/kg-day of N-nitrosodimethylamine in gelatin capsules with dextrose vehicle to groups of 6 or 18 beagle dogs on two consecutive days per week for up to 24 weeks. Treatment was discontinued during weeks 5-7 and 18-19 when the most severely afflicted dogs were removed from treatment, based on clinical signs and clinical chemistry scores. At resumption of treatment, dose levels were individually adjusted based on a physical examination and laboratory tests. Clinical laboratory tests were repeated 3 and 4 weeks after the final dosing, at which time the dogs were euthanized, necropsied and part of the liver was processed for histopathological examination. All dogs responded to N-nitrosodimethylamine, but variably, so that the severity of hepatic toxicity did not precisely correlate with duration of treatment. Only one treated dog was euthanized in a moribund condition. The following effects were observed in a majority of treated dogs: increased postprandial bile acid levels, weight loss, increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, high serum ALP activity, high GGT activity, increased sulfobromphthalein retention, low serum cholesterol and total protein concentrations, elevated blood ammonia level, low serum albumin concentration, prolonged partial thromboplastin and activated prothrombin times, low blood urea nitrogen (BUN) levels, ascites accumulation, low antithrombin III activity, and low packed cell volume. The following effects were observed in less than a third of the treated dogs: high bilirubin levels, icterus, gastrointestinal hemorrhage, and hepatic encephalopathy. Relative liver weights were lower in treated dogs and hepatic pathology included necrosis, inflammation, cholestasis, fibrosis, biliary hyperplasia, and lobular collapse. The dose level of 2 mg/kg-day was a FEL for severe hepatic effects and weight loss.

Butler-Howe et al. (1993) administered a similar regimen of 0 or 2 mg/kg-day of N-nitrosodimethylamine in gelatin capsules with dextrose to groups of 6 or 10 dogs on two consecutive days per week for up to 56 weeks. In addition to physical examination and clinical chemistry analyses, dogs were given abdominal ultrasonographic and hepatic scintigraphic examinations to evaluate the development of portosystemic shunts (anomalous connections between the hepatic portal vein and the posterior vena cava and its tributaries). Clinical signs of hepatic dysfunction in treated dogs included ascites, intermittent anorexia, weight loss and icterus. Treatment-related signs of hepatic disease included a 13- to 54-fold increase in bile acid concentrations, elevated ammonia levels and sulfobromphthalein retention times (20- to 40-fold increase), and significantly reduced serum albumin concentrations. N-nitrosodimethylamine induced multiple portosystemic shunts in all treated dogs: in eight dogs, within 8 to 14 weeks of treatment and in two dogs, within 40 to 56 weeks. In addition, the number and size of hepatic and portal veins within the hepatic parenchyma was reduced in treated dogs. Histologically, treated dogs exhibited moderate to severe diffuse, chronic hepatic fibrosis (9/10), hepatic atrophy and regeneration (8/10), and moderate to severe centrilobular necrosis (5/10). None of these lesions were observed in control dogs. Gross lesions observed in treated dogs at necropsy included moderately firm livers (10/10), hepatic fibrosis (9/10), reduced liver size (9/10), and cirrhosis (9/10). The 2 mg/kg-day exposure level was a FEL for severe hepatic effects in dogs.

In a study of immunological effects, Desjardins et al. (1992) exposed groups of 10-15 outbred female CD-1 mice to 0, 1, 5, 10, or 20 ppm of N-nitrosodimethylamine in drinking water for 0, 30, 60, 90, or 120 days. The intake of N-nitrosodimethylamine was reported to be 0, 0.01, 0.05, 0.1, or 0.5 μg/kg-day. However, these doses, as reported, were not consistent with other mouse drinking water studies (e.g., Anderson et al., 1978, 1986) or with mouse reference values that have been developed by U.S. EPA. Based on reference body weight and water consumption values (U.S. EPA, 1988), the intakes of N-nitrosodimethylamine in this study were calculated as 0, 0.26, 1.3, 2.6, and 5.3 mg/kg-day. These values are four orders of magnitude greater than the doses reported in the study and are consistent with the rest of the data base. Mortality and the accumulation of ascites were monitored throughout the study. At the end of each treatment, mice were immunized by intraperitoneal injection of sheep red blood cells (SRBC). After 4 days, serum was collected for agglutinin testing (quantified by plaque forming units, PFU), mice were sacrificed and the spleens were harvested for evaluation of cell-mediated immune response by the mixed lymphocyte reaction (MLR) test. For the MLR test, spleen lymphocytes from N-nitrosodimethylamine-exposed CD-1 mice were cultured with mitomycin-C treated stimulatory cells from female C57B1/6 mice and radiothymidine incorporation was monitored five days later.

N-nitrosodimethylamine exposure resulted in dose-related effects on mortality associated with ascites accumulation (Desjardins et al., 1992). At the 20 ppm level, one mouse died and the remainder exhibited ascites by 30 days of exposure; the authors discontinued treatment and further analysis at this level because of the severity of hepatotoxicity. At the 10 ppm level, there was one death during the course of the study, but an increasing number of animals were afflicted with ascites as the study duration continued: 1/13, 3/13, 5/13, and 10/13 at 30, 60, 90, and 120 days, respectively. No ascites or mortality was observed at the 0-5 ppm exposure levels. Individual variability in the primary humoral response to SRBC was observed in control and experimental groups, possibly related to the genetics of the outbred strain. Nevertheless, the humoral response (anti-SRBC antibody response) was significantly suppressed in the 5 ppm group after 90 days and in the 10 ppm groups after 90 or 120 days of treatment, although it was significantly increased in the 10 ppm group after 30 days. Similarly, treatment with 5 or 10 ppm for 90 or 120 days significantly suppressed the cellular immune response. Exposure at 1 ppm had no effect on humoral or cellular immune responses. In a parallel experiment, the humoral immunity assay was performed on groups of 10-15 mice that were exposed to 0, 5, or 10 ppm of N-nitrosodimethylamine for 90 days and maintained for another 30 days without treatment. The immunosuppression observed at 90 days was reversed in the 5 ppm group, but persisted in the 10 ppm group despite the month-long withholding of treatment. This study identified a subchronic NOAEL of 1 ppm (0.26 mg/kg-day) and a LOAEL of 5 ppm (1.3 mg/kg-day) for immunosuppression in mice. The NOAEL and LOAEL doses are estimates based on reference values because these values are much more consistent with the rest of the data base than the extremely low values reported, without any supporting details, by the researchers. The large discrepancy between the doses reported by the researchers and those estimated from reference values was a source of uncertainty in this study.

Chronic Studies

Terao et al. (1978) fed a basal diet containing 10 ppm of N-nitrosodimethylamine to 15 male Wistar rats for 54 weeks and fed them an unadulterated diet for an additional 15 week observation period. Using reference values for body weight and food consumption (U.S. EPA, 1988), the intake of N-nitrosodimethylamine was calculated as 0 or 0.74 mg/kg-day. A group of 30 control rats received the basal diet for the entire 69 weeks. After five weeks, one animal per group was sacrificed for histopathology and electron microscopy. At termination, all animals were necropsied, the major organs (unspecified) were examined for histopathology and the livers were examined for ultrastructural changes by transmission electron microscopy. No body weight or food consumption data were reported. Treatment with N-nitrosodimethylamine had no significant effect on survival. Ultrastructurally, the liver of the single animal examined at five weeks showed a marked proliferation of smooth endoplasmic reticulum. However, at termination, the livers of treated rats were reported as having "almost normal" histology. No hepatic carcinoma was observed in the control or treatment group, but 7/15 treated rats developed Leydig-cell tumors of the testes, compared to 0/30 controls. This study did not provide any useful information for noncancer risk assessment, because of the lack of data on body weight or noncancer lesions. In addition, it is likely that adverse hepatic effects may have been partially reversed during the 15-week observation period.

Terracini et al. (1967) fed groups of 6-19 male Porton rats diets containing 0, 2, or 5 ppm of N-nitrosodimethylamine and groups of 5-62 female rats 0, 2, 5, 10, 20, or 50 ppm for 52 weeks, after which they were maintained without treatment for up to week 104 or 120. Using reference values for Fischer 344 rats (U.S. EPA, 1988; data on Porton rats were not available, and Terracini et al. did not report body weights), these food concentrations were estimated to provide doses of about 0, 0.2, or 0.4 mg/kg-day for males and doses of 0, 0.2, 0.4, 0.8, 1.6, or 4.0 mg/kg-day for females. At death, rats received a complete necropsy, including histological examination, if possible. Body weight data were not reported. Hepatic cysts that appeared to replace the parenchyma occurred in the livers of rats treated with at least 5 ppm; cysts were stated to be unrelated to tumors when they occurred in the same liver. No other treatment-related nonneoplastic lesions were reported. Hepatic tumors developed in male rats at levels of 2 or 5 ppm and in females at 5 ppm or above. Lung tumors were observed in male rats at 2 ppm and in female rats at 2 and 5 ppm. This study is not suitable for derivation of an RfD because the year-long observation period provided ample time for recovery of any reversible effects.

Arai et al. (1979) fed groups of male and female Wistar rats (18-24 per gender per group) 0, 0.1, 1.0, or 10 ppm of N-nitrosodimethylamine in the diet for 96 weeks. Body weight and water and food consumption were recorded weekly. Using these data presented graphically, the intake of N-nitrosodimethylamine was calculated to have been 0, 0.012, 0.125, or 1.23 mg/kg-day in males and 0, 0.024, 0.24, or 2.4 mg/kg-day in females. At termination, survivors were subjected to a complete necropsy. Blood samples were analyzed for hematological and clinical chemistry parameters (red and white blood cell count, hematocrit, hemoglobin, AST, ALT, ALP, choline esterase, total protein, and BUN). All major organs were examined, and liver, kidneys, and spleen were weighed and analyzed for histopathology.

N-nitrosodimethylamine had no clear effect on the general condition, food or water consumption, or on body weight of exposed rats (Arai et al., 1979). In all groups of males, body weights showed a slight decline after week 68; however, there was no clear dose-relationship. Treatment had no effect on kidney weights, but resulted in >60% increases in liver weight and an 8-fold increase in spleen weight in high-dose females, as well as a 2-fold increase in spleen weight in high-dose males. The authors attributed the increased spleen weight to the development of leukemia in this group. Hematological evaluation revealed a 20% reduction in erythrocyte counts and 35% increase in leukocyte counts in high-dose females. N-nitrosodimethylamine had no significant effect on clinical chemistry. Pyelonephritis (inflammation of the kidney parenchyma and lining of the renal pelvis) was reported in all groups (30% in controls of both genders and in treated females; >50% in low- and mid-dose males), but was highest in the high-dose males (80%). Liver tumors (nodular hyperplasia, cancer, hemangioendothelioma, fibrosarcoma) developed at dietary levels at or above 1 ppm (0.125 or 0.24 mg/kg-day in males and females, respectively), but not at 0.1 ppm (0.012 or 0.024 mg/kg-day in males and females, respectively). This study was not useful for noncancer risk assessment, as observed changes occurred at doses that produced tumors and appeared to have been secondary to tumor development.

In another cancer study, Peto et al. (1991) exposed groups of 6-week-old male and female Colworth (Wistar-derived) rats (60/gender/group) to 15 concentrations between 0.033 and 16.896 ppm of N-nitrosodimethylamine in drinking water (distilled). As calculated from water intake data provided graphically, the intakes of N-nitrosodimethylamine ranged between 0.0015-0.79 mg/kg-day in males and 0.0025-1.25 mg/kg-day in females. Groups of 240 male and 240 female rats received distilled water as controls. The rats were exposed for a lifelong maximum of ~3.5 years, except for two groups of 6 rats from each dose group that were scheduled for sacrifice after one year or after 18 months of exposure. The endpoints that were examined weekly included body weight, water intake, and palpable liver abnormalities; however, body weight data were not reported. Rats with "clearly palpable" liver abnormalities were sacrificed and necropsied for gross lesions and the liver, esophagus, kidneys, bladder, lungs, skull, and tissues with gross lesions were examined for histopathology. Exposure to levels at or above 0.528 ppm reduced survival in both genders because of a higher incidence of hepatic cancers. The authors reported significant dose-related trends (measured across all 15 dose groups) for the following nonneoplastic lesions of the liver: hyperplastic nodules, cytomegaly, shrinkage of hepatocytes, abnormality of glycogen-containing cells, and cysts.

Several of these lesions were clearly related to the carcinogenic effects of N-nitrosodimethylamine. Hyperplastic nodules likely represent preneoplastic effects and many of the cysts that were observed were reported to be due to preneoplastic lesions of the blood vessels. The tumor-related reductions in survival in animals exposed to concentrations at or above 0.528 ppm limit the value of data on nonneoplastic lesions above this level. No significant change (from control values) in the incidence of the remaining nonneoplastic lesions (cytomegaly, hepatocyte shrinkage, and abnormality of glycogen-containing cells) was observed at concentrations below 0.528 ppm (based on Fisher's exact tests conducted for this review). Finally, the toxicological significance of the remaining lesions was uncertain. The importance of the lesion characterized as "abnormality of glycogen-containing cells" was unclear. It was

difficult to reconcile the meaning of dose-related increases in both cytomegaly (cell growth) and cell shrinkage. However, only the cytomegaly appeared to exhibit a positive dose-response trend.

Reproductive/Developmental Studies

Napalkov and Alexandrov (1968) administered single doses of 30 mg/kg-day N-nitrosodimethylamine (purity not specified) by gavage to groups of 6 pregnant white non-inbred rats. The authors indicated that the date of conception was verified, but did not note how. Each group was treated on a different gestational day, and results were recorded between the 17th and 21st gestation days. Embryos and placentas were examined grossly and microscopically; further details of the examinations were not provided. The authors reported a notable increase in embryolethality when N-nitrosodimethylamine was administered on the third gestational day and indicated that the highest mortality occurred in the groups treated on the 10th or 12th gestational day. Based on graphical representation of the results, it appeared that embryo mortality was approximately 35% on day 3, 60% on day 10, and 40% on day 12, compared with a control incidence of about 10% on all days. In experiments with pregnant rats treated at 1 mg/rat/day for the first or second week of gestation, embryo mortality was reported to be 37-40%; the incidence of embryo deaths in controls was not reported. The authors reported "no lethal effect" in animals treated during the final week of gestation. Treatment throughout pregnancy with daily doses of 0.5 mg/rat resulted in 20% mortality of embryos. The authors indicated that no teratogenic effects were observed in any animals treated with N-nitrosodimethylamine. Maternal effects of treatment were not reported, although the authors reported that treatment with single intraperitoneal or intravenous doses resulted in "similar toxic effects on mothers" as the oral doses. The authors noted that the doses used were slightly below the LD_{50}. This study had a number of significant shortcomings. Apart from the information provided above, no other details of experimental design or findings were provided. Further, it was not clear whether control groups were used for all experiments. Based on the single-dose experiments, the 30 mg/kg dose appeared to be a FEL for embryolethality. The lack of data on controls in the other experiments precluded the identification of effect levels from these experiments.

In a follow-up study, Alexandrov (1973) administered a single gavage dose of 0 or 30 mg/kg N-nitrosodimethylamine (purity not specified) in saline to groups of at least 6 pregnant noninbred albino rats on the 3rd, 9th, 10th, or 12th days of gestation. Although the report was not clear, it appears that the dams were sacrificed between the 17th and 21st days of gestation. The authors reported that the "ordinary method of teratologic investigation" was used but did not detail the evaluations conducted. The authors indicated that there were no teratogenic effects in experiments with N-nitrosodimethylamine; however, the nature of the teratogenicity evaluations was not reported. No information on maternal effects was reported; embyrolethality was the only finding reported. Based on graphical representation of the data, the embryo mortality rates were approximately 5% for controls and 31%, 19%, 42%, and 27% for treatment on gestation day 3, 9, 10, and 12, respectively. The 30 mg/kg dose from this study was a FEL for embryolethality.

Alexandrov (1973) conducted an additional experiment in which pregnant rats were given a single gavage dose of 30 mg/kg on the 21st gestational day. Offspring were followed

until their natural death and tumor formation was recorded. No information on nonneoplastic endpoints was reported.

Bhattacharyya (1965) exposed pregnant albino Wistar rats to N-nitrosodimethylamine in the diet. Upon sacrifice (the date of which varied by group), the abdomens were examined and livers of both dams and fetuses were fixed for histopathology. Two rats were given diets containing 200 ppm and six rats were given 100 ppm "from early in pregnancy." Another group of 4 rats was treated at 50 ppm from the first day of pregnancy through one month postpartum. It did not appear that control animals were used in the study. Based on reference values for food consumption and body weight (U.S. EPA, 1988), these concentrations result in doses of about 5, 10, and 20 mg/kg-day. No information on maternal effects other than mortality was provided. One of the two rats treated at 200 ppm died on gestation day 19. Four rats treated at 100 ppm were sacrificed on GD 20 and had resorbed all of their fetuses, leading to the sacrifice of the remaining two rats. The authors reported that only a few fetuses from the remaining two animals treated at 100 ppm survived. Histological examination of the fetal livers revealed abnormalities in only one (the number of fetal livers examined was not reported) in which plasma cells, neutrophils, eosinophil macrophages, and lymphocytes were found near portal and hepatic veins. Among the fetuses of dams treated at 50 ppm, there were no histopathological findings in the livers examined "at frequent intervals." No other information was provided on results in the group treated at 50 ppm. The lack of controls, coupled with the limited reporting of study design and findings, precludes the identification of effect levels in this study.

Nishie (1983) dosed groups of 6-14 pregnant Holtzman rats with 0, 15, or 20 mg/kg of N-nitrosodimethylamine in olive oil via gavage on gestational day (GD) 7, 10, 12, 13, 14, 16, or 18. For comparison, a set of nonpregnant female rats was also treated once at the same dose levels. Two days after treatment, blood samples were harvested for clinical chemistry (glucose, BUN, triglyceride, cholesterol, inorganic phosphorus, total protein and ALP, AST, ALT, creatinine phosphokinase, and α-hydroxybutyric dehydrogenase activities). Pregnant rats were sacrificed on GD 15-20. Dams were weighed before necropsy and fetal weights were recorded. Liver tissue taken from dams was assayed for ascorbic acid content. Maternal liver, kidneys, and adrenal and thyroid glands were weighed and examined for histopathology. The severity of N-nitrosodimethylamine effects on dams varied with gestational day. N-nitrosodimethylamine treatment before GD 18 had no effect on mortality of dams, but administered on GD 18 increased mortality by 9.4% at the low dose and 35.3% at the high dose. N-nitrosodimethylamine administered on GD 15 or 20 produced statistically significant decreases in maternal body weight. Treatment with either dose significantly increased the numbers of mitotic cells in the liver and adrenal cortex, relative adrenal weights, serum AST and serum ALT activities, and decreased liver ascorbic acid and total serum protein levels in dams. Both doses resulted in severe hepatic effects (severe centrilobular damage, glycogen depletion) in dams, but had no effect on thyroid histology. The only fetal effect reported was a statistically significant reduction in fetal body weight following maternal treatment on GD 15 or 20. The low dose in this study, 15 mg/kg-day, was a FEL for increased mortality and severe hepatic effects in pregnant rats.

In a preliminary experiment, Anderson et al. (1978) first administered 0 or 0.1 ppm of N-nitrosodimethylamine in drinking water to groups of 10 female CD-1 mice for 10 weeks.

Based on water consumption data provided (6.2 mL/day) and a reference body weight of 24.6 g (U.S. EPA, 1988), the intake of N-nitrosodimethylamine was approximately 0.025 mg/kg-day. Females were then mated and continued treatment during pregnancy. Treatment with N-nitrosodimethylamine had no significant effect on the number of females with litters at weaning (9/10 vs 10/10 control) or on the total number of offspring per group (74 vs. 100 control). No other information was provided on the results of this experiment.

In the main experiment, Anderson et al. (1978) gave groups of 20 female CD-1 mice 0 or 0.1 ppm of N-nitrosodimethylamine in drinking water for 75 days and then mated; treatment resumed when the pregnant females were placed in individual cages. Water consumption, weight gain, conception time, litter size, rates of stillbirth and neonatal death, weaning weight, gender ratio of offspring, and histopathology of major organs of dead pups were evaluated. Treatment with N-nitrosodimethylamine had no effect on preconception weight gain or water consumption (data not reported), conception rate, duration of gestation, total number of offspring born, litter size, or weight of weanlings. However, 10% of treated offspring were stillborn and another 10% died within two days after birth; the total 20% death rate for offspring was significantly larger than the 9.9% rate in controls. The difference between the two groups was primarily due to the increased rate of stillbirths in the treated group (19/185 vs. 5/182); the difference in neonatal deaths was much smaller (19/185 vs. 13/182). Although the number of litters with offspring deaths was slightly higher in the treated group (11/20 = 55%) than in controls (8/20 = 40%), the difference was not statistically significant. This indicates that the overall increase in offspring deaths in the treated group was due to an increase in the number of deaths within litters that had at least one death. There was one litter, of unspecified size, which was composed entirely of dead offspring. This litter by itself could have accounted for the difference between treated and control groups. Therefore, it was not clear that the overall increase in dead offspring observed in this study represents a treatment-related effect. In fact, the control group in a third experiment described in the same report (but not involving N-nitrosodimethylamine) and conducted in a similar fashion to the second experiment had a combined stillborn and neonatal death rate of 14/101 = 14%, which was not significantly different from the N-nitrosodimethylamine treated group in the main experiment ($p=0.1$ by Fisher Exact Test conducted for this review).

Histological examination of stillborn fetuses and dead neonates showed that death occurred before breathing commenced, but revealed no other abnormalities; no effect was observed on the fetal liver (Anderson et al., 1978). Weanlings of treated dams included twice as many males (n=101) as females (n=51), which was significantly different from the more equal gender ratio of controls (80M, 84F). According to the authors, the disproportionate number of males did not result from excess mortality among the female neonates. In the preliminary experiment, there was only a slight excess of males at weaning (40M, 34F) that did not differ from controls (49M, 51F). Although the smaller preliminary study did not indicate as pronounced a difference in gender distribution, the significant excess of males among weanlings in the main experiment may represent a developmental effect of N-nitrosodimethylamine. The dose of 0.025 mg/kg-day in this study was considered a LOAEL for possible effects on weanling gender ratio and perinatal mortality.

Inhalation Exposure

The available studies in animals examined carcinogenicity, but not most nonneoplastic effects, following chronic inhalation exposure to N-nitrosodimethylamine.

Klein et al. (1989, 1991) exposed groups of 36 female Sprague-Dawley rats by inhalation to 0, 0.04, 0.2, or 1 ppm (0, 0.12, 0.6, or 3 mg/m^3) of N-nitrosodimethylamine 4 hours/day, 5 days/week over a period of 502 days and observed them for life. The authors estimated that the exposure corresponded to an intake of 0, 0.01, 0.04, or 0.18 mg/kg-day. Tumor-bearing animals were sacrificed and organs examined for histopathology. Animals in all treatment groups had an increased incidence of nasal tumors that were not observed in controls. No data regarding nonneoplastic lesions were reported. At the 1 ppm level, survival was significantly reduced by nine months compared to the control and 0.2 ppm groups; however survival in the 0.04 ppm group was about two months longer than in controls. Body weight data presented only graphically suggested a consistent dose-related trend for reduced body weight among exposed rats (see Figure 1). Although weight differences from controls appeared to exceed 10% only at the highest dose (1 ppm; 3 mg/m^3; ~0.18 mg/kg-day) until rats in the lower dose groups were older than three years of age, when very few animals remained alive, the dose-related trends appeared to be significant, suggesting a minimal LOAEL of 0.12 mg/m^3 (0.04 ppm; ~0.01 mg/kg-day) for reduction in body weight.

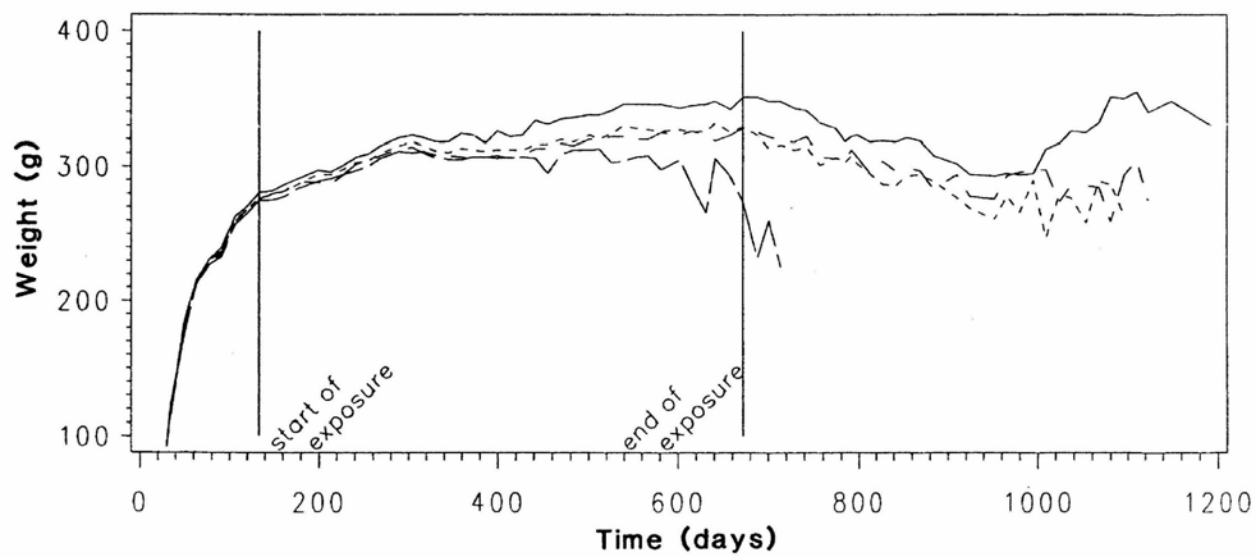

Figure 1. Body weight of rats exposed by inhalation to N-nitrosodimethylamine at 0 (–), 0.04 ppm (---), 0.2 ppm (- -), and 1.0 ppm (—-) (Klein et al., 1991).

Moiseev and Benemanskii (1975) reported that they continuously exposed groups of 31-36 male and 30-51 female Wistar rats by inhalation to 0.005 or 0.219 mg/m^3 of N-nitrosodimethylamine for 25 months. Control rats (40 male and 37 female) were maintained under ordinary conditions. Every three months, 4-6 animals per group were sacrificed for

histological analysis. Neither mortality nor body weight data were reported. An increased incidence of tumors in kidney, liver, and lungs was observed in high-exposure rats of both genders. Tumor incidence in the low exposure rats was observed to be similar to controls. No data were reported regarding the incidence of nonneoplastic lesions. A similar experiment in which groups of 33-47 male and 30-68 female Balb/c mice were exposed to the same concentrations for 17 months produced comparable results.

Other Studies

In a study on the transplacental carcinogenicity of N-nitrosodimethylamine, Anderson et al. (1989) exposed pregnant C3H/HeNCr MTV mice to a single intraperitoneal dose of either 37 or 7.4 mg/kg on GD 16 or 19. The animals were observed daily and killed when moribund, at which time they were subjected to complete necropsy, primarily aimed at identifying and quantifying tumors. The authors reported that a dose of 37 mg/kg on either day resulted in the deaths of 3/3 litters exposed. No other information was presented on effects at this dose. Although detailed results were not provided, it appears that there were no effects on perinatal mortality, nor was gender ratio affected, at 7.4 mg/kg-day (48 M and 63 F offspring from dams treated on GD 16; 68 M and 55 F from those treated on GD19).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR N-NITROSODIMETHYLAMINE

No quantitative data were available that reported health effects in humans following long-term oral exposure to N-nitrosodimethylamine. The developmental drinking water study in CD-1 mice (Anderson et al., 1978) was chosen as the critical study for the subchronic and chronic RfD, with support from the Peto et al. (1991) liver toxicity study. Anderson et al. (1978) identified a free-standing LOAEL of 0.025 mg/kg-day in mice for developmental effects, including possible effects on weanling gender ratio and perinatal mortality. Potential points of departure are summarized in Table 2. Benchmark dose modeling of the data in this study was not possible, as only a single dose group was used. Other limitations of the study included incomplete reporting of results and uncertainty as to whether the perinatal mortality was attributable to treatment or other causes. However, the effect of treatment on weanling gender ratio was pronounced (101M, 51F) and, coupled with a possible effect on perinatal viability, provided a reasonable basis for the p-RfD. Further, the absence of histopathology findings in the livers of the dead pups in this study indicated that a p-RfD based on the developmental study should be somewhat protective for liver effects.

The long-term chronic drinking water study by Peto et al. (1991) focused on cancer, but also reported data for several noncancer liver toxicity endpoints. This study had a number of strengths, such as starting with more than 5000 experimental animals and considering sixteen dose levels with doses above and below apparent NOAELs. This assessment concluded that only three of the reported endpoints were unrelated to neoplasia. Of these nonneoplastic endpoints, only liver cell cytomegaly exhibited a reasonable dose-response trend. Although the study also reported the opposite effect, hepatocyte shrinkage, the dose-response curve for this

Table 2. Potential Points of Departure from Studies of Ingestion of N-nitrosodimethylamine in Water

Citation	Endpoint	Animal	Duration	LOAEL (ppm) [a]	LOAEL (mg/kg-day) [b]	NOAEL (ppm) [a]	NOAEL (mg/kg-day) [b]	UF	Potential p-RfD (mg/kg-day)
Desjardins et al., 1992	Immuno-suppression	Mice	90-120 [c]	5	1.3 [d]	1	0.26 [e]	3000[f]	**$9x10^{-5}$**
Anderson et al., 1978 [g]	>conception time [c] >stillbirths [h] >neonatal death [h] >male births [i]	Mice-Female	~100 days [j]	0.1	0.025	- - - - -	- - - - -	3000[k]	**$8x10^{-6}$**
Anderson et al., 1978 [g]	Developmental liver pathology	Mice-Female	~100 days [j]	- - - - -	- - - - -	0.1	0.025 [l]	- - - -	- - - - - -
Peto et al., 1991	Liver cell cytomegaly	Rat-Male	3.5 years	1.056	0.043	0.528	0.022	300[m]	**$7x10^{-5}$**

[a] PPM data as reported in studies.
[b] Except for the Desjardins et al. data, NOAELs and LOAELs in mg/kg-day are as reported or calculated using consumption data provided in studies
[c] Average conception time among treated mice reported as 3 days longer than controls; insufficient data provided to determine if this was a significant difference.
[d] Calculated using EPA (1988) default values for body weight and water consumption; study reported dose of $5x10^{-5}$ mg/kg-day.
[e] Calculated using EPA (1988) default values for body weight and water consumption; study reported dose of $1x10^{-5}$ mg/kg-day.
[f] Uncertainty Factor calculations: UF_A=10; UF_H=10; UF_D=3 (developmental data; data for 2 species); subchronic point of departure (POD) UF=10
[g] Single dose with controls, only.
[h] Some question as to whether doubled perinatal mortality was due entirely to treatment.
[i] Twice as many male weanlings as females, even in litters with low perinatal mortality.
[j] Dosing for at least 75 days preconception and continued until births
[k] Uncertainty Factor calculations: UF_A=10; UF_H=10; UF_D=3 (developmental data; data for 2 species); UF_L=10 (3, if "minimal" LOAEL)
[l] Not a true NOAEL; other effects were observed.
[m] Uncertainty factor calculations: UF_A = 10; UF_H = 10; UF_D = 3 (developmental data for 2 species)

endpoint was relatively flat, suggesting that this response might have been unrelated to dosing with N-nitrosodimethylamine.

Benchmark dose modeling of the cytomegaly data (Peto et al., 1991) indicated a reasonable dose-response curve, but suggested $BMDL_{10}$ points of departure that were higher than the apparent LOAEL. BMD models with the best AIC values also calculated $BMDL_5$ values above this LOAEL, though some models with reasonably low AICs calculated $BMDL_5$ values below the LOAEL. Based on these observations, we concluded that attempting to predict a point of departure from BMD data would require potentially arbitrary assumptions. The apparent NOAEL for liver cell cytomegaly in male rats was 0.022 mg/kg-day, almost identical to the LOAEL chosen as the point of departure for reproductive and developmental effects in the Anderson et al., 1978 study.

The subchronic drinking water study by Desjardins et al. (1992) reported immunosuppression in CD-1 mice exposed at or above doses estimated in this assessment to be 1.32 mg/kg-day, but not at 0.26 mg/kg-day. At higher doses (2.6 mg/kg-day and higher), there was mortality and many of the survivors developed severe hepatotoxicity (as manifested by ascites). The authors examined only gross pathology and did not conduct any histopathology examinations.

Other subchronic studies in animals demonstrated that oral exposures above 1 mg/kg-day adversely affect the liver, resulting in necrosis, cirrhosis, reduced body weight and, frequently, increased mortality (rats: Khanna and Puri, 1966; Barnes and Magee, 1954; Jenkins et al., 1985; Nishie, 1983; mice: Takayama and Oota, 1965; Anderson et al., 1986; dogs: Boothe et al., 1992; Butler-Howe et al., 1993). In most of these studies, the lowest tested dose represented a FEL; thus, these studies were not considered for use in RfD development. Barnes and Magee (1954), and Anderson et al. (1986) each identified a freestanding LOAEL of at least 5 mg/kg-day. Anderson et al. (1986) used only a single dose, while all of the animals treated by Barnes and Magee (1954) at doses higher than the LOAEL died prior to study termination; thus benchmark dose modeling could not be performed on the data in either study. Chronic oral studies in rats, which used doses between ~1 μg and ~1 mg/kg-day, reported leukemia or cancer of the liver or testes (Peto et al., 1991; Arai et al., 1979; Terao et al. 1978). All chronic studies focused on carcinogenicity and, other than Peto et al. (1991), none was considered useful for noncancer dose-response assessment.

Although hepatotoxicity endpoints from N-nitrosodimethylamine exposures were well-supported in the literature, including qualitative human data, the rat developmental data (Anderson et al., 1978) were chosen as identifying the point of departure for deriving the p-RfD. These developmental data indicated a freestanding LOAEL similar to the NOAEL for hepatic cytomegaly reported by Peto et al. (1991). This suggested that developmental effects might occur at dose levels below those leading to hepatotoxicity, leading to the conclusion that this uncertainty must be accounted for in calculating the p-RfD.

The freestanding LOAEL of 0.025 mg/kg-day for developmental effects (Anderson et al., 1978), was used as the point of departure for calculating the p-RfD. The following uncertainty factors were selected:

- 10 for animal to human extrapolation (UF_A)
- 10 to protect sensitive human subpopulations (UF_H)
- 10 for using a LOAEL as the point of departure (UF_L)
- 3 for database uncertainties (UF_D), including absence of neurotoxicity and multigenerational reproductive studies

for a composite UF of 3000.

With the exception of the database UF, these represent EPA default values for uncertainty. Whether the point of departure was a minimal LOAEL was considered, since there were questions about both developmental effects reported: the cause of the excess perinatal mortality was unclear and the potential mechanism of action for the severely altered weanling gender ratios was unknown. However, because perinatal mortality might have resulted from the dosing, the full UF of 10 was retained for this uncertainty. Also considered was whether the database uncertainty factor should be increased to 10. However, because data were available in both mice and rats (Peto et al., 1991) and because the critical study (Anderson et al., 1978) was a developmental study, based on standard EPA methodology, a database UF of 3 was considered sufficient.

The freestanding LOAEL of 0.025 mg/kg-day identified in the developmental toxicity study by Anderson et al. (1978) was used to calculate a **provisional subchronic and chronic oral RfD** for N-nitrosodimethylamine of $8x10^{-6}$ mg/kg-day, as follows:

Subchronic and chronic p-RfD	=	LOAEL ÷ UF
	=	0.025 mg/kg-day ÷ 3000
	=	**0.000008 or $8X10^{-6}$ mg/kg-day, 8E-6 mg/kg-day**

Other potential points of departure considered, and resulting p-RfDs are listed in Table 2.

Confidence in the critical study is low. Adequate numbers of mice were used, but the study included only a single exposure level and results were not reported completely. Further, neither the perinatal mortality nor the unusual but marked effect on the weanling gender ratio had been confirmed in other developmental studies of N-nitrosodimethylamine. Confidence in the resulting RfD can be increased somewhat by noting that Peto et al. (1991) reported data with an apparent NOAEL similar to the Anderson et al. (1978) LOAEL selected as the POD.

Three older studies report mortality in offspring of pregnant rats orally exposed to N-nitrosodimethylamine (Alexandrov, 1974; Napalkov and Alexandrov, 1968; Bhattacharyya, 1965). However, these studies used doses approaching the LD_{50} for N-nitrosodimethylamine, and results were poorly reported, with little or no information on maternal toxicity. Anderson et al. (1989) did not observe perinatal mortality in the offspring of mice given a single intraperitoneal dose of 7.4 mg/kg-day during gestation. It was difficult to draw comparisons among the available developmental toxicity studies, because only Anderson et al. (1978) exposed animals prior to conception as well as during gestation. Confidence in the databases for both subchronic and chronic toxicity is low. Most subchronic studies employed dose levels that had

frank toxic effects on the liver, while data from chronic studies assessing carcinogenicity did not generally address nonneoplastic effects at low exposures (less than 1 mg/kg-day). There were no systematic studies of neurotoxicity and the database lacks a multi-generation reproductive toxicity study. Low confidence in the RfD follows.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR N-NITROSODIMETHYLAMINE

Provisional inhalation RfCs could not be derived for N-nitrosodimethylamine because toxic effects data were insufficient. Furthermore, in the absence of pharmacokinetic data and because portal of entry effects could not be ruled out, route to route extrapolation from the oral p-RfD could not be justified. However, the Klein et al. (1989, 1991) inhalation cancer study reported data on survival and body weight that were considered of sufficient basis for a screening p-RfC. However, the Appendix of this document contains a Screening Value that may be useful in certain instances. Please see the attached Appendix for details.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR N-NITROSODIMETHYLAMINE

Weight-Of-Evidence Classification

IRIS (last revised in 1993) provides a classification of B2 (probable human carcinogen) under 1986 Guidelines for Carcinogen Assessment.

Quantitative Estimates of Carcinogenic Risk

No quantitative values are developed because IUR and OSF are available on IRIS.

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APPENDIX

DERIVATION OF A SCREENING VALUE FOR N-NITROSODIMETHYLAMINE

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for N-nitrosodimethylmine, chronic RfC. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "Screening Value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. In the OSRTI hierarchy, Screening Values are considered to be below Tier 3, "Other (Peer-Reviewed) Toxicity Values."

Screening Values are intended for use in limited circumstances when no Tier 1, 2, or 3 values are available. Screening Values may be used, for example, to rank relative risks of individual chemicals present at a site to determine if the risk developed from the associated exposure at the specific site is likely to be a significant concern in the overall cleanup decision. Screening Values are not defensible as the primary drivers in making cleanup decisions because they are based on limited information. Questions or concerns about the appropriate use of Screening Values should be directed to the Superfund Health Risk Technical Support Center.

Available inhalation data were insufficient to derive an inhalation p-RfC. However, the following proposes derivation of a screening inhalation RfC, using the Klein et al. (1989, 1991) data described in the PPRTV manuscript.

Body weight data presented only graphically (Figure 1) suggested a consistent dose-related trend for reduced body weight among exposed rats. Weight differences from controls appeared to exceed 10% only at the highest dose (1 ppm = 3 mg/m^3; ~0.18 mg/kg-day) until rats in the lower dose groups were older than three years of age, when very few animals remained alive. However, these small dose-related trends in weight appeared to show significant differences between rats in the low dose group (0.12 mg/m^3 = 0.04 ppm; ~0.01 mg/kg-day) and controls, suggesting a minimal LOAEL of 0.12 mg/m^3 for reduced body weight. However, quantitative data were insufficient to formally test significance.

The POD of 0.12 mg/m^3 was used to calculate a screening p-RfC, applying the following uncertainty factors:

- UF_a = 10, using animal data as the POD
- UF_h = 10, protection of sensitive human populations
- UF_d = 10, inhalation data only in rats and only for weight and survival
- UF_L = 3, the LOAEL was considered minimal, because body weight reductions were very small in exposed rats

Chronic, screening inhalation p-RfC = 0.12 mg/m^3 / 3000 = **$4x10^{-5}$ mg/m^3, 4E-5 mg/m^3**

This screening inhalation toxicity value is very uncertain because the data reported did not include weights of individual animals and were insufficiently quantitative to permit statistical tests of the weight differences or trends. However, this screening value might be supported by the similarity of the estimated equivalent inhalation daily dose at the point of departure, estimated by Klein et al. (1989, 1991) to be 0.01 mg/kg-day for reduced body weight in rats, with the oral LOAEL POD of 0.025 mg/kg-day for developmental effects in mice, used to derive the oral p-RfD.