9-19-2007

Provisional Peer Reviewed Toxicity Values for

N-Nitrosodiphenylamine
(CASRN 86-30-6)

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
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
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-NITROSODIPHENYLAMINE (CASRN 86-30-6)

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 new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. 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, 1993) did not list a RfD or RfC for N-nitrosodiphenylamine. No RfD for N-nitrosodiphenylamine was listed in the Drinking Water Regulations and Health Advisories list (U.S. EPA, 2006). In addition, no RfD or RfC was listed in the HEAST (U.S. EPA, 1997). The CARA database (U.S. EPA, 1991, 1994) listed two potentially relevant documents, a Health and Environmental Effects Profile (HEEP) for nitrosamines (U.S. EPA, 1986), and a Health Effects Assessment (HEA) for N-nitrosodiphenylamine (U.S. EPA, 1987). Neither document calculated a RfD for N-nitrosodiphenylamine because of its oral carcinogenicity in rodents. Similarly, an ATSDR (1993) Toxicological Profile did not derive a chronic oral MRL for N-nitrosodiphenylamine because the critical noncancer endpoints identified by ATSDR (urinary bladder hyperplasia and metaplasia) in a chronic rat bioassay (NCI, 1979) were considered to be preneoplastic. Neither the HEEP nor the HEA contained information regarding inhalation toxicity of N-nitrosodiphenylamine. ATSDR did not derive inhalation MRLs for N-nitrosodiphenylamine due to the absence of reliable inhalation data. Standards or guidelines relating to occupational inhalation exposure to N-nitrosodiphenylamine were not listed by the ACGIH (2007), NIOSH (2005), or OSHA (2006). However, for the important metabolite, diphenylamine, AGGIH listed a TLV-TWA of 10 mg/m^3, based on liver, kidney, and blood toxicity; and NIOSH recommended a REL-TWA of 10 mg/m^3.

Both a cancer classification and an oral slope factor for N-nitrosodiphenylamine were available on IRIS (U.S. EPA, 1993). The cancer assessment classified N-nitrosodiphenylamine in category B2 (probable human carcinogen) under 1986 Guidelines for Carcinogen Assessment, based on bladder tumors in rats of both genders, reticulum cell sarcomas in mice, and the structural similarity of the chemical to other carcinogenic nitrosamines. IRIS (U.S. EPA, 1993) also reported an oral slope factor of 4.9 E-3 per mg/kg-day based on transitional cell carcinomas of the bladder in female Fischer 344 rats reported by NCI (1979). No quantitative estimate of cancer risk from inhalation exposure was available on IRIS.

Other resources examined included an Environmental Health Criteria document on nitroso compounds (WHO, 1978), an IARC (1982) monograph, and the NTP (2006) database. No information regarding noncancer toxicity of N-nitrosodiphenylamine was located in Patty's Industrial Hygiene and Toxicology (Lijinsky, 2001). Literature searches of TOXLINE (1981-1993), TSCATS, RTECS, and HSDB were conducted and screened in May 1993; TOXLINE and TSCATS were updated in November 2000, and updated 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

No data were located regarding the toxicity of N-nitrosodiphenylamine to humans following chronic or subchronic exposure by any route.

Animal Studies

Oral Exposure

NCI (1979) conducted long-term carcinogenicity studies of N-nitrosodiphenylamine in F344 rats and $B6C3F_1$ mice that also examined some systemic toxicity endpoints, which were considered for RfD derivation. No other studies of non-neoplastic endpoints were identified in the literature, although the NCI data also were summarized in Cardy, et al., 1979.

In order to identify maximum tolerated doses (MTDs) for the chronic studies, NCI (1979) administered N-nitrosodiphenylamine (98% pure) in the diet to F344 rats and B6C3F1 mice (5/gender/species) for 8 weeks (male and female mice and female rats) or 11 weeks (male rats). Concentrations of N-nitrosodiphenylamine in the diet ranged from 1000 to 46,000 ppm. NCI (1979) did not report food consumption. Using default reference values for body weight and food consumption (U.S. EPA, 1988), we estimated corresponding doses from 100 to 4600 mg/kg-day in rats and from 180 to 8300 mg/kg-day in mice. Body weights were recorded twice each week; animals were sacrificed under carbon dioxide and necropsied upon study termination. Details of the pathology examinations were not provided. Three of the five female rats exposed to ~1600 mg/kg-day died prior to scheduled sacrifice. All female rats treated with higher doses also died. Terminal body weights were reduced by 10% or more in male and female rats exposed to concentrations of at least ~400 mg/kg-day , in male mice exposed to at least ~1700 mg/kg-day, and in female mice exposed to either ~3700 mg/kg-day or ~8300 mg/kg-day. Female mice treated at intermediate concentrations did not exhibit significantly reduced body weights. NCI (1979) did not provide details of the pathology findings in the subchronic studies, except to note that the only histopathological finding was trace pigmentation of Kupffer's cells in the livers of male mice exposed to ~8300 mg/kg-day N-nitrosodiphenylamine.

In the chronic studies, groups of 50 male and 50 female F344 rats were fed 1000 or 4000 ppm N-nitrosodiphenylamine (98% pure) in the diet for 100 weeks (NCI, 1979). Controls

consisted of 40 rats (20/gender) fed the basal diet. NCI did not report food consumption for this study. Using default reference values for body weight and food consumption (U.S. EPA, 1988), we estimated the administered doses to be 80 or 300 mg/kg-day for males and 90 or 400 mg/kg-day for females. Animals were observed twice daily and weighed monthly, except during weeks 38-68 (reason not provided); moribund animals were sacrificed. Clinical examinations were conducted monthly. Comprehensive macroscopic and microscopic examinations of major organs and all gross lesions were conducted on animals that died and on surviving animals at study termination.

In the male rats, survival at study termination was comparable between groups (NCI, 1979). Survival was 16/20 (80%), 44/50 (88%) and 43/50 (86%), for the control, low- and high-dose groups, respectively. In female rats, there was a statistically significant decrease in survival only in the high-dose group, with 18/20 (90%), 44/50 (88%) and 35/50 (70%) of the control, low- and high-dose groups surviving until study termination. Nearly all deaths of high-dose females occurred during the last 15 weeks of the study and might have reflected tumor-related mortality.

From data presented graphically (NCI, 1979), a dose-related depression in body weight was apparent in the high-dose male rats throughout the study and in low-dose males after week 70; weight was not measured for weeks 38-68. Body weights in treated males were approximately 5% and 13% lower than controls during weeks 70-101 for the low- and high-dose groups, respectively. Dose-related depression of body weight also became apparent in treated females after week 70, with the low-dose group showing an approximate 7% decrease and the high dose group showing about an 18% decrease.

NCI (1979) reported that corneal opacity was observed in 15/50 high-dose male rats (0/20 in controls) and 16/50 low-dose females (1/20 in controls). NCI (1979) did not report incidences at other doses, the wording of the document implied that it was not observed in low-dose males or high-dose females. The report did not state when, during the course of the study, these lesions were noted. The opacity was discussed as a “clinical sign” and presumably was detected during the monthly clinical examinations. The eye was not included in the list of tissues examined for histopathology. No information was presented as to the nature or severity of the opacities seen. NCI (1979) reported that corneal opacity might have been related to N-nitrosodiphenylamine treatment. However, the absence of corneal opacities in high-dose females, despite the high incidence in low-dose females, suggested that these opacities were not related to chemical treatment.

The only finding reported by NCI (1979) as non-neoplastic that was dose-related and not generally found in untreated aging F344 rats was an increased incidence of epithelial hyperplasia of the bladder in male (0/19, 2/46, 6/45) and female (0/18, 4/48, 7/49) rats, summarized in Table 1. Although pairwise comparisons of these data using Fisher’s exact test (conducted for this review) did not indicate a significant change from control values, a Cochran-Armitage trend test for the male rat data was significant at $p<0.05$. The trend in females was marginally significant ($p<0.10$). As described below and summarized in Table 1, similar rates of bladder epithelial hyperplasia were observed in mice treated at much higher doses.

Table 1. Incidence of bladder epithelial hyperplasia in rodents fed N-Nitrosodiphenylamine in diet (NCI 1979)

	Rats			Mice		
	Control (mg/kg-day)	**Low dose (mg/kg-day)**	**High dose (mg/kg-day)**	**Control (mg/kg-day)**	**Low dose (mg/kg-day)**	**High dose (mg/kg-day)**
Males	0/19 (0)	2/46 (4%) (80)	6/45 (13%) (300)	0/18 (0)	2/49 (4%) (1700)	7/46 (15%) (3400)
Females	0/18 (0)	4/48 (8%) (90)	7/49 (14%) (400)	0/18 (0)	3/49 (6%) (~400)	5/38 (13%) (~1000)

The US EPA National Center for Environmental Assessment commissioned reviews of the NCI (1979) rat bladder epithelial hyperplasia data, by three independent pathologists, to assist in determining the suitability of this endpoint for use in deriving a p-RfD (Sciences International, 2007). In response to EPA questions, the three pathologists each concluded that the rat bladder epithelial hyperplasia was preneoplastic, indicating the hyperplasia had preceded and in some cases evolved into carcinoma. NCI (1979) descriptions of microscopic observations of rat bladders specified two features of the lesions that had been seen only in malignant neoplasms, anaplasia and invasion. In addition, previous population studies with rats chronically administered the urothelial carcinogen N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) demonstrated that hyperplastic lesions were highly prevalent with early exposure, prior to the appearance of carcinomas (Akagi et al., 1973; Ito et al., 1969). The architecture of epithelial hyperplasia in the NCI (1979) study could be subdivided into lesions that conformed to the overall structure of the underlying bladder, flat lesions, and those that began to form connective tissue stroma and appeared papilloma-like, papillary lesions (Sciences International, 2007). The acinar type structures, noted in some cases, often have been identified in conjunction with neoplastic changes in human bladders rather than reactive or inflammatory conditions. The pathology reviewer concluded that at least the subset of flat lesions that contained these acinar-type structures most likely were associated with early neoplastic transformation of the urothelium and that the papillary-type lesions described in the NCI (1979) study appeared to represent primary neoplasms of the bladder. In further support of this view, the degree of mitotic activity, atypia, and sheet-like growth pattern suggested that at least a proportion of these lesions conformed to current nomenclature for high-grade papillary urothelial carcinomas. In the rat model, the increased rates of hyperplasia seen in the high-dose paradigm were paralleled by the development of invasive urothelial carcinoma, which supported this conclusion. In contrast, similar rates of urothelial hyperplasia were present in mice treated with N-nitrosodiphenylamine, although only rare invasive urothelial carcinomas appeared to arise in this model system, which may have represented differences in molecular mechanisms underlying tumor progression in these model systems.

NCI (1979) reported neoplasms in rat bladder transitional cells and referred to the hyperplasia as non-neoplastic lesions in the bladder epithelium. However, transitional cells form the mucosal lining of the urinary bladder and serve as the bladder epithelium. NCI reported that

in high-dose groups of each gender of rats, "the entire spectrum from transitional-cell hyperplasia to transitional-cell carcinoma was observed in the urinary bladder." Based on these observations and conclusions of the pathologists who independently reviewed these data (Sciences International, 2007), the bladder epithelial hyperplasia appeared to have been preneoplastic rather than non-neoplastic.

Two of the three pathologist reviewers concluded that the NCI (1979) preneoplastic hyperplasia was a non-threshold response resulting from mutagenicity; the third expert pathology reviewer concluded the preneoplastic hyperplasia was a threshold response, resulting from irritation with consequent regenerative proliferation, and was not due to mutagenicity. This reviewer supported his conclusion that the bladder epithelial hyperplasia was a threshold response by making the following points:

- Bladder hyperplasia and neoplasia clearly were due to irritation with consequent regenerative proliferation
    - Irritation resulted from cytotoxic stimulus
    - Cytotoxic stimulus probably was urinary tract calculi, or other solids
- Mutagenic MOA was not possible based on chemistry & metabolism of N-nitrosodiphenylamine & metabolites
    - N-nitrosodiphenylamine does not have an alpha-carbon available for hydroxylation
    - Possibility of transnitrosation seemed highly unlikely
- N-nitrosodiphenylamine dose required for hyperplasia or tumors was extremely high compared to N-nitrosamines that have produced bladder cancer in rats, such as N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)
    - Hyperplastic lesions were observed in mice, without tumors, even though the dose was much higher in mouse than in the rat
    - Rat and mouse have appeared to be similarly susceptible to other N-nitrosamines, as demonstrated with BBN, with the mouse possibly being somewhat more susceptible
    - Nonmutagenic chemicals that act via toxicity and regeneration tend to be more active in rats than mice
- Lack of evidence of an inflammatory reaction in rats might have been because the inflammation might have resolved by the time of examination
- Chronic inflammation or chronic infection with schistosomes appeared to contribute directly to the pathogenesis of some bladder cancers, such as squamous cell carcinomas

A reviewer who concluded that the neoplastic urothelial hyperplasias most likely resulted from a non-threshold mutagenic response also noted that in a subset of bladder cancers, such as squamous cell carcinomas, chronic inflammation or chronic infection with schistosomes appeared to contribute directly to the pathogenesis of these tumors. However, this reviewer also pointed out that, although chronic submucosal inflammation was a striking finding in a large number of mice treated with N-nitrosodiphenylamine (Table 2), few cases ultimately progressed to invasive urothelial carcinoma. The reviewers who concluded the hyperplasia was a nonthreshold response also supported their position with the following points:

- Progressive molecular alterations arising from changes at the genetic level generally have been understood to cause neoplastic changes independent of associated inflammation (Wu, 2005)
- Few cases of mouse submucosal inflammation progressed to invasive urothelial carcinoma
- Rates of urothelial hyperplasia did not vary tremendously between rat and mouse models, despite markedly higher level of chronic inflammation in the mouse
- Urothelium typically undergoes morphologic changes associated with chronic irritation, most notably squamous metaplasia.
    - Unlike hyperplastic changes, squamous metaplasia is a relatively stable change.
    - Incidence of squamous metaplasia in the NCI study was extremely low (only 3 cases)
    - Incidence of squamous metaplasia should be higher in a threshold response where only a subset of chronically injured bladders would progress to carcinoma.
- Scarring, chronic inflammation, & other signs of chronic injury were not mentioned as features in the bladders of N-nitrosodiphenylamine treated rats

The pathologist reviewers (Sciences International, 2007) disagreed regarding the likelihood that the rat bladder preneoplastic hyperplasia, reported by NCI (1979) was a threshold response, resulting from inflammation, or a non-threshold response, resulting from mutagenicity. Because of the uncertainty about the MOA leading to these lesions, the default view of a non-threshold linear response was considered to apply to these data.

The 300 and 400 mg/kg-day doses in male and female rats were considered to be LOAELs for decreased (13-18%) body weight, based on positive trend test (NCI, 1979). The body weight depression observed in the rats was consistent with the reported depression of body weight gain in dogs following chronic ingestion of diphenylamine, a metabolite of N-nitrosodiphenylamine (Thomas et al., 1967; U.S. EPA, 2007). In rats, N-nitrosodiphenylamine apparently is denitrosated to diphenylamine and nitric oxide (Appel et al., 1984). The low dose of 80-90 mg/kg-day in male and female rats was a NOAEL for weight gain depression in this study. Body weight changes at this dose were only 5-7%, the incidence of bladder hyperplasia was 4-8%, and the lack of dose-response in corneal opacities suggested that the observed opacities were not chemical-related.

NCI (1979) reported a variety of neoplastic lesions in the rats, most notably urinary bladder transitional cell carcinomas that were observed in 16/45 high-dose males, and in 40/49 high-dose females; no equivalent carcinomas were observed in control or low-dose rats of either gender. Among the high-dose rats exhibiting these carcinomas, one of the males and two of the females had a squamous metaplasia. While only one carcinoma appeared to have metastasized, the carcinoma growth patterns were described by NCI (1979) as "quite large", and might have been the cause of death in many animals

In the mouse portion of the chronic bioassay (NCI, 1979), groups of 50 male $B6C3F_1$ mice were fed 10,000 or 20,000 ppm of N-nitrosodiphenylamine (98% pure) in the diet for 101 weeks. Female $B6C3F_1$ mice (50/treatment group) initially were fed 5000 or 10,000 ppm in the diet. This regimen in females was stopped at 38 weeks due to severely decreased body weight

gain relative to controls in both treated groups. At 41 weeks, dietary concentrations were modified to 1000 or 4000 ppm and treatment continued for the following 60 weeks. NCI calculated the time-weighted average dietary concentrations for the females to be 2315 or 5741 ppm for the experiment. Controls consisted of 20 mice/gender fed the basal diet for 101 weeks. NCI (1979) did not report food consumption for this study, making interpretation of weight gain data difficult. Using default reference values for body weight and food consumption (U.S. EPA, 1988), we estimated administered doses to be 1700 or 3400 mg/kg-day for the males, and an average of 400 or 1000 mg/kg-day for the females. Animals were observed twice daily and weighed monthly; moribund animals were sacrificed. Clinical examinations were conducted monthly. Comprehensive macroscopic and microscopic examinations of major organs and all gross lesions were conducted on animals that died and on surviving animals at study termination.

With the exception of high-dose females, mouse survival was comparable between the control and treatment groups. In males, 18/20 (90%), 46/50 (92%) and 41/50 (82%) of the controls, low- and high-dose animals, respectively, lived until the end of the study. Survival in females at study termination was 16/20 (80%) for the control, 42/50 (84%) for the low-dose, and 31/50 (62%) for the high-dose groups. Tarone tests conducted by NCI (1979) demonstrated no significant dose-related trend.

Mice of both genders showed a dose-related decrease in body weight that persisted throughout the study, according to data presented only in graphical form (NCI, 1979). Body weights were approximately 12% and 22% lower in treated males than in controls for weeks 50-101 in the low- and high-dose groups, respectively. After week 50, treated females had gained approximately 36% and 49% less weight than controls in the low-dose and high-dose groups, respectively.

Bladder lesions occurred with higher frequency in treated mice of both genders than in their respective controls. Chronic submucosal inflammation of the bladder was increased in a dose-dependent fashion in both male and female mice (See Table 2). While there were transitional cell carcinomas (one in each gender of the low-dose group), papillomas (one in each low- and high-dose group of males), a hemangioma, and bladder epithelial hyperplasia (9 cases in each gender of treated groups), NCI (1979) did not consider these treatment-related. Perivascular lymphocytic cuffing in the kidney also was reported in both genders of mice, but the incidence of these lesions was not dose-related and did not correlate with the changes in the urinary bladder. The female mice were more susceptible to the adverse bladder effects of N-nitrosodiphenylamine at high doses. A NOAEL was not identified in this study.

Several chronic studies of N-nitrosodiphenylamine in rodents focused on tumor induction (BRL, 1968; Innes et al., 1969; Druckrey et al., 1967; Argus and Hoch-Ligeti, 1961). Experimental details of systemic toxicity (e.g., body weight, non-neoplastic lesions) or of controls in these studies either were not provided or were inadequate to assess noncancer endpoints.

No data were identified regarding potential adverse developmental effects from exposure to N-nitrosodiphenylamine. However, Crocker, et al. (1972) noted renal effects in rats following

Table 2. Incidence of Bladder Submucosal Inflammation in Mice Fed Diets Containing N-Nitrosodiphenylamine (NCI, 1979)

	Control	Low dose	High dose
Male	0/18[a] (0 mg/kg-day)	12/49[b] (24%) (1700 mg/kg-day)	31/50[c] (62%) (3400 mg/kg-day)
Female	0/18[a] (0 mg/kg-day)	31/47[c] (66%) (~400 mg/kg-day)	30/38[c] (79%) (~1000 mg/kg-day)

[a] $p<0.0001$ by Cochran-Armitage trend test performed for this review
[b] $p<0.05$ by Fisher Exact test performed for this review
[c] $p<0.0001$ by Fisher Exact test performed for this review

maternal ingestion of the N-nitrosodiphenylamine metabolite, diphenylamine. Similar effects have been observed in diphenylamine-dosed adult hamsters (Lenz et al., 1995), mice (Rohrbach et al., 1993), chickens (Sorrentino et al., 1978), dogs (Thomas et al., 1967), rats, and gerbils (Lenz and Carlton, 1990).

Inhalation Exposure

A 20-day rat inhalation study (Zhilova and Kasparov, 1966) was cited by ATSDR (1993). However, ATSDR did not consider this study to be reliable due to inadequate reporting of experimental methods and results. No other studies were located regarding inhalation toxicity of N-nitrosodiphenylamine in animals.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC OR CHRONIC ORAL RfD VALUES FOR N-NITROSODIPHENYLAMINE

No information regarding adverse health effects in humans following oral exposure to N-nitrosodiphenylamine were available. The available subchronic toxicity study in animals was not of adequate quality for deriving a subchronic p-RfD. Specifically, the subchronic portion of the study conducted by NCI (1979) was aimed at identifying the maximum tolerance dose (MTD). Small numbers of animals were used (5/gender/species) and few details of the pathology examinations and findings were presented.

The NCI (1979) chronic dietary study in rats and mice was chosen as the critical study to attempt to derive a chronic p-RfD. This study used adequate numbers of animals and provided adequate dose-response information; however, the study was limited by a lack of data on hematology and clinical chemistry, and incomplete reporting of data relating to noncancer endpoints. The variation in dosing of the female mice was especially problematic, since these animals seemed to be most sensitive to adverse bladder effects from ingestion of N-nitrosodiphenylamine.

The NCI (1979) chronic rat study identified a LOAEL of 300-400 mg/kg-day for decreased body weight gain (13-18%) in male and female rats, with a NOAEL of 80-90 mg/kg-

day. However, the validity of significantly decreased body weight gain as a critical effect was questionable because food consumption was not reported; the weight deficits might have resulted from reduced food consumption due to palatability. In female rats at the high dose of 400 mg/kg-day, there was a tumor-related reduction in survival. The low dose was considered a minimal LOAEL for bladder epithelial hyperplasia in male and female rats. However, the pathology review commissioned by NCEA (Sciences International, 2007) concluded these lesions were preneoplastic. One reviewer specifically concluded that the hyperplasia was a threshold response due to irritation with a consequent regenerative proliferation, and was not a mutagenic response, although no data for N-nitrosodiphenylamine, *per se*, specifically supported this view. However, the other two pathology reviewers concluded it probably was a non-threshold response; one pathologist specifically indicated the response resulted from mutagenicity.

Based on the unanimous conclusion that the hyperplasia was preneoplastic and the disagreement regarding whether it was a threshold response, we concluded this endpoint was inappropriate for use as a critical effect to derive a p-RfD. Thus, the only potential POD for deriving a p-RfD from the NCI (1979) rat data was the NOAEL of 80-90 mg/kg-day for depression of weight gain. However, this potential POD was questionable because it might have resulted from reduced food consumption rather than toxicity.

In the mouse study, NCI (1979) reported large increases in the incidence of chronic submucosal inflammation of the bladder (Table 2) at both dose levels and dose-related depression of body weight gains in mice of both genders exposed to N-nitrosodiphenylamine in the diet. Although similar effects were seen in both genders, the effective doses were much lower and incidence of inflammation higher in females. The study identified no NOAEL for bladder inflammation or depression of body weight in mice. These data indicated that bladder submucosal inflammation in female mice was the most sensitive endpoint from which to derive a potential p-RfD for N-nitrosodiphenylamine. However, the female mouse data were extremely problematic. During the first 38 weeks of treatment, female mice were fed approximately 900 or 1800 mg/kg-day and exhibited severely depressed body weight gains, suggesting the maximum tolerated dose (MTD) might have been exceeded. As a result, NCI (1979) apparently stopped dosing these animals for three weeks and then resumed feeding at approximately 180 or 700 mg/kg-day for the remaining 60 weeks of the study. Based on NCI (1979) calculated average concentrations in feed and default values for food ingestion and body weight (US EPA, 1988), we estimated time-weighted average doses for the female mice to be approximately 400 or 1000 mg/kg-day. This resulted in a nominal LOAEL of approximately 400 mg/kg-day for bladder submucosal inflammation in female mice. The very high response rate of 66% suggested that a point of departure for this inflammatory effect should be substantially lower. Consequently, we conducted benchmark dose modeling of these data (Appendix A), calculating a BMD_{10} of 25 mg/kg-day and a $BMDL_{10}$ of 17 mg/kg-day.

All quantal models in U.S. EPA's Benchmark Dose Software (BMDS) were fit to the incidence data for bladder submucosal inflammation in female mice. The default BMR of 10% increase in extra risk was used for this analysis. Models were run using the default restrictions on parameters built into the BMDS. Appendix A contains a summary of the modeling results and a plot of the best fitting model. Adequate fits (goodness of fit p-value >0.10) were achieved

by multiple models, however, the log-logistic model provided the best fit, as indicated by lowest Aikaike Information Criterion (AIC). However, the female mouse BMD of 25 mg/kg-day and BMDL of 17 mg/kg-day appeared unreliable because of the questionable shape of the BMDR curve and the fact that these values were based on a lowest incidence rate of 66%, resulting in a BMDL 24 times lower than the lowest experimental data.

In addition to these concerns about the reliability of the BMD analysis, were concerns resulting from the severe weight gain depression exhibited by the female mice during the early high-dose period, suggesting that these doses might have approached or exceeded the maximum tolerated dose. In addition, the dose calculations were extremely uncertain because of the lack of food consumption data, variation in N-nitrosodiphenylamine feed concentrations, and cessation of exposure for three weeks during which the female mouse treatment groups apparently received no N-nitrosodiphenylamine.

Considering these problems, we concluded that confidence in the mouse data from the critical chronic oral study (NCI, 1979) was too low to use for derivation of a p-RfD. While the study used adequate numbers of animals, it did not include evaluation of hematology or clinical chemistry, featured only limited reporting of noncarcinogenic endpoints, and identified neither a NOAEL nor a reasonable LOAEL in mice. Especially problematic was the dosing regimen for the most sensitive species, female mice, which was changed during the course of the study due to significant effects on body weight gain. The rat body weight gain NOAEL of 80 – 90 mg/kg-day also was considered an inappropriate POD, because it clearly was not the most sensitive endpoint and might have resulted from reduced food consumption rather than toxicity. Finally, the observation of corneal opacity in certain treatment groups of rats was an insufficient endpoint because the effect apparently was not related to dose.

Consequently, the poor quality of the available data, the availability of an IRIS oral slope factor addressing the cancer and hyperplasia risk observed in rats, and major uncertainties in dosing and modeling results led us to conclude that data were insufficient to derive either a chronic or a subchronic p-RfD for N-nitrosodiphenylamine.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC OR CHRONIC INHALATION RfC VALUES FOR N-NITROSODIPHENYLAMINE

A provisional inhalation RfC could not be derived for N-nitrosodiphenylamine because data on adverse health effects following inhalation exposure were lacking for humans and animals. Without pharmacokinetic data and information to rule out portal-of-entry effects, there was no basis to support a route-to-route extrapolation from the oral data, even if it were otherwise considered sufficient.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR N-NITROSODIPHENYLAMINE

Weight-of-Evidence Descriptor

The IRIS cancer assessment (U.S. EPA, 1993), classified N-nitrosodiphenylamine in category B2, probable human carcinogen, using the 1986 Guidelines for Carcinogen Assessment. This classification was based on the NCI (1979) data on bladder tumors in rats (both genders), reticulum cell sarcomas in mice, and the structural similarity of the chemical to other carcinogenic nitrosamines. These data seemed consistent with the equivalent descriptor of "Likely to be carcinogenic to humans" in the updated U.S. EPA (2005) guidance.

Quantitative Estimate of Carcinogenic Risk

IRIS (U.S. EPA, 1993) reported an oral slope factor of 4.9×10^{-3} per mg/kg-day, based on transitional cell carcinomas of the bladder in female F344 rats reported by NCI (1979). No quantitative estimate of cancer risk from inhalation exposure was available on IRIS. None was developed here due to lack of information.

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APPENDIX A

RESULTS OF BENCHMARK DOSE MODELING FOR CHRONIC ORAL DOSING

Table A-1. BMD Modeling Results for Female Mouse Submucosal Inflammation of Bladder (NCI, 1979)

Model	Degrees of Freedom	χ^2	χ^2 Goodness of Fit p-Value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Log-logistic (slope ≥ 1)	2	0.26	0.8783	101.65	25	17
Log-probit (slope ≥ 1)	2	3.31	0.1912	104.44	90	70
Gamma (power ≥ 1)	2	4.14	0.1265	105.30	52	41
Multistage (degree=1)[b]	2	4.14	0.1265	105.30	52	41
Quantal Linear	2	4.14	0.1265	105.30	52	41
Weibull (power ≥ 1)	2	4.14	0.1265	105.30	52	41
Logistic	1	11.64	0.0006	119.03	128	100
Probit	1	11.90	0.0006	119.34	128	104
Quantal Quadratic	1	17.23	0.0000	125.26	250	197

[a]Adequate fit indicated by p>0.10
[b]Model shown is lowest degree polynomial providing adequate fit. Betas restricted to ≥ 0.

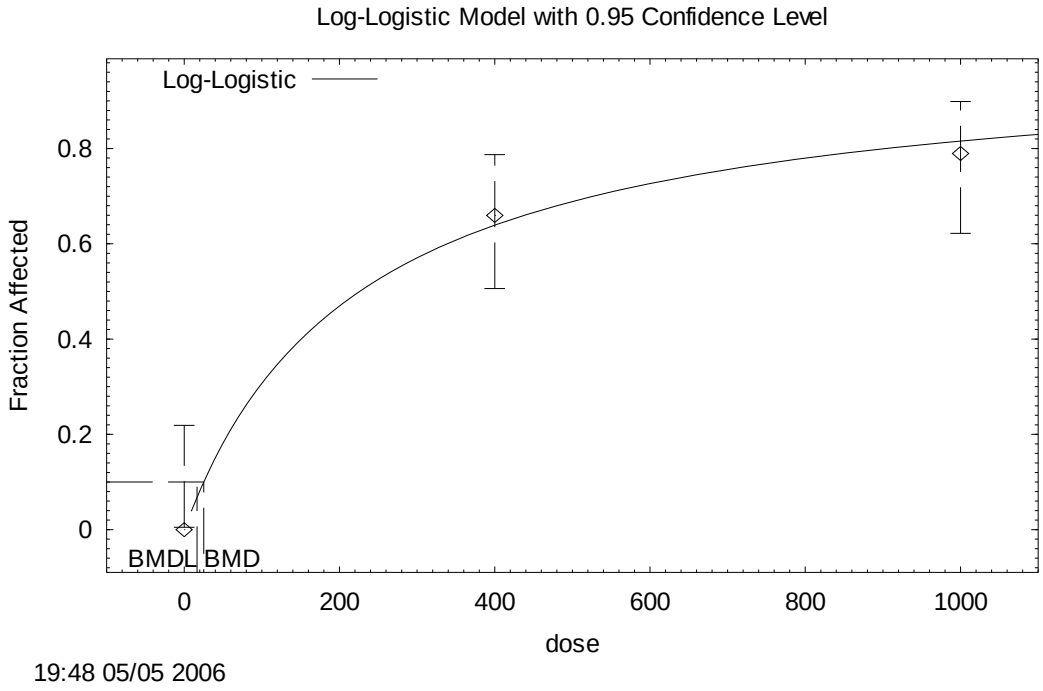

Figure A-1. Log-logistic BMD_{10} Model Fit to Incidence Data for Submucosal Inflammation of Bladder in Female Mouse (NCI, 1979)