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Provisional Peer-Reviewed Toxicity Values for

2-Nitroaniline
(CASRN 88-74-4)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
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
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
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2-NITROANILINE (CASRN 88-74-4)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.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. U.S. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. 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 U.S. EPA's 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 U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. 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 5-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 documents 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 Resource Conservation and Recovery Act (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 document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. 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 U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

2-Nitroaniline is an intermediate in azo dyes (Benya and Cornish, 1994). It is an orange massive solid at room temperature, commercialized as flakes, or melted above 71°C, with a purity >99.6%. The empirical formula for 2-nitroaniline is $C_6H_6N_2O_2$ (see Figure 1). It has a molecular weight of 138.1, a density of 1.442 g/cm^3, a vapor pressure of 0.00368 hPa at 25°C, a boiling temperature of 284°C and a water solubility of 1170 mg/l at 20°C.

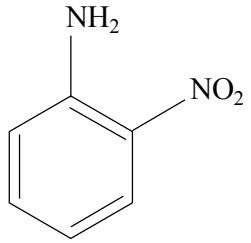

Figure 1. 2-Nitroaniline Structure

The U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2008) does not list a chronic oral reference dose (RfD), a chronic inhalation reference concentration (RfC), or a cancer assessment for 2-nitroaniline. 2-Nitroaniline is not listed on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The HEAST (U.S. EPA, 1997) does not include subchronic or chronic RfD values for 2-nitroaniline, but it does list a subchronic RfC of 2E-03 mg/m^3 and a chronic RfC of 2E-04 mg/m^3 for 2-nitroaniline, which are derived in a Health and Environmental Effects Document (HEED; U.S. EPA, 1991a) for 2-Nitroaniline. The RfC values are based on a LOAEL of 9.8 mg/m^3 in male rats (Bio/Dynamics, 1983b) and include uncertainty factors (UFs) of 1000 (10 for

extrapolation from animal data, 10 for sensitive individuals, and 10 for the use of a LOAEL) for the subchronic RfC and 10,000 (including an additional UF of 10 for the use of a subchronic study) for the chronic RfC. Although the critical effect listed in the HEAST is hematological effects, the derivation in the source HEED is actually based on nasal irritation (LOAEL 9.8 mg/m^3; NOAEL not identified), because hematological effects were only observed at higher concentrations. However, hematological effects are used as the critical effect for derivation of subchronic and chronic oral RfD values in the HEED by route-to-route extrapolation from the inhalation data. In addition to the HEED, a Health and Environmental Effects Profile (HEEP; U.S. EPA, 1985) for Nitroanilines (*o*-, *m*-, *p*-) was also listed as a reference for the assessment in the HEAST, but it does not derive toxicity values for 2-nitroaniline, citing inadequate data. The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991b, 1994a) includes no other relevant documents for 2-nitroaniline.

No standards for occupational exposure to 2-nitroaniline have been established by the ACGIH (2006), NIOSH (2006), or OSHA (2006). The ATSDR (2006) and the WHO (2006) have not published toxicological reviews on nitroanilines or 2-nitroaniline. The HEAST (U.S. EPA, 1997) does not list a cancer assessment for 2-nitroaniline. IARC (2006) and the NTP (2005) have not evaluated the carcinogenicity of 2-nitroaniline. We also consulted toxicity reviews on aromatic nitro, amino, and nitro-amino compounds (Weisburger and Hudson, 2001; Woo and Lai, 2001) for relevant information.

Literature searches for studies relevant to the derivation of provisional toxicity values for 2-nitroaniline (CASRN 88-74-4) were conducted in MEDLINE, TOXLINE special, and DART/ETIC (1960s−August 2006); BIOSIS (2000−August 2006); TSCATS/TSCATS2, RTECS, CCRIS, HSDB, and GENETOX (not date limited); and Current Contents (April 2008−September 2008).

REVIEW OF PERTINENT DATA

Human Studies

No studies were located regarding the effects of subchronic or chronic exposure of humans to 2-nitroaniline by oral or inhaled routes.

Animal Studies

Oral Exposure

Limited data are available regarding the toxicity of 2-nitroaniline to laboratory animals by repeated oral exposure. A summary of available animal studies is provided in the Table 1.

Table 1. Summary of Oral Toxicity Studies

Study	Species	Duration	Doses	Critical Effect	NOAEL	LOAEL	Comments
14-day rat study (Komsta et al., 1989)	SD[a] rats, 10/sex/group	14 days	0, 1, 10, 100 mg/kg-day, gavage	No	Free-standing 100 mg/kg-day	No	
Rat developmental range finding (Monsanto Co., 1984)	CD rats, 6/group	GD[b] 6−15	0, 50, 200, 400, 800, or 1200 mg/kg-day in corn oil, gavage	Maternal: reduced body weight, food consumption and clinical signs Fetal: reduced fetal weight	400 mg/kg-day	800 mg/kg-day	Same NOAEL for both maternal and fetal effects
Rat developmental (Monsanto Co., 1985)	CD rats, 25/group	GD 6−15	0, 100, 300, or 600 mg/kg-day in corn oil, gavage	Maternal: piloerection, pale or cold extremities Fetal: situs inversus	300 mg/kg-day	600 mg/kg-day	Same NOAEL for both maternal and fetal effects
Rat developmental (Sisti, 2001a)	SD rats, unknown sample size	GD 0−19	0, 100, 200, or 400 mg/kg-day in polyethylene glycol, gavage	Maternal: matted fur, and piloerection	Maternal: 200 mg/kg-ay Fetal: 400 mg/kg-day	400 mg/kg-day	
Rat reproductive (Sisti, 2001b)	SD rats, 12/sex/group	9 weeks, starting 4 weeks before mating, females were treated up to 4 days after delivery	0, 50, 150, or 450 mg/kg-day in PEG, gavage	Maternal: reduced body weight gain, clinical signs Fetal: increased mortality, reduced body weight	150 mg/kg-day	450 mg/kg-day	Same NOAEL for both maternal and fetal effects

[a]SD = Sprague-Dawley
[b]GD = Gestation Day

A 14-day toxicity study conducted by Komsta et al. (1989) evaluated the effects of repeated oral exposure to 2-nitroaniline in rats. Groups of 10 male and 10 female young adult Sprague-Dawley rats, weighing 200−300g, were administered 2-nitroaniline (purity 97−99%) in corn oil at doses of 0, 1, 10, or 100 mg/kg-day by gavage. Animals were weighed and evaluated daily for clinical signs. At the end of treatment, blood samples were analyzed for hematology, and clinical chemistry and hepatic microsomal enzyme activities were assessed. Necropsy was performed, organ weights were recorded, and histopathologic examination was conducted on 29 tissues (including spleen, liver, and bone marrow). No mortalities, clinical signs, or treatment-related changes in body weight were observed throughout the study. All hematology parameters (hematocrit [Hct], hemoglobin [Hgb], mean cell hemoglobin [MCH], and erythrocyte and leukocyte counts) in treated animals were comparable to controls; methemoglobin was not measured. No effects were observed on clinical chemistry, organ weights (brain, heart, kidney, liver, and spleen), hepatic microsomal enzyme activities, gross pathology, or histopathology. The highest dose of 100 mg/kg-day was identified as a free-standing NOAEL in this study.

Developmental toxicity studies have been conducted by Monsanto Co. (1984, 1985) and Sisti (2001a,b). The studies by Sisti (2001a,b) were identified in the Screening Information Data Set on 2-nitroaniline prepared by the Organization for Economic Cooperation Development (OECD/SIDS, 2003); however, these studies are unpublished and efforts to obtain complete study reports were not successful. The summary provided in this PPRTV document is based on only the information obtained from OECD/SIDS report (2003).

Developmental studies conducted by Monsanto Co. consisted of a range-finding study (Monsanto Co., 1984) and a main study (Monsanto Co., 1985). In the range-finding study, groups of six mated female Charles River CD rats were administered 2-nitroaniline (99.8% pure) in corn oil at doses of 0, 50, 200, 400, 800, or 1200 mg/kg-day by gavage on gestational days (GD) 6−15 (Monsanto Co., 1984). Mortality and clinical signs were evaluated daily; body weights were recorded at several intervals throughout the study. Dams were sacrificed on GD 21 for gross necropsy and examination of uterine content. Live fetuses were examined for sex, weight, and external malformations. The authors did not analyze the maternal blood for hematology parameters and histopathologic examinations were not performed. In the 1200-mg/kg-day group, 4/6 rats died between GD 8−11; no deaths were observed in other treatment groups (Monsanto Co., 1984). Prior to death, animals exhibited hypoactivity, convulsions, prostration, salivation, piloerection, shallow respiration, and loss of muscle coordination. On necropsy, subcutaneous, and abdominal fat of rats in this dose group was colored yellow, indicating deposition of the test material. In rats receiving ≥800 mg/kg-day, body weight gain, and food consumption were reduced (statistical analysis not performed by study authors), and clinical signs were observed: dyspnea, incoordination, and lethargy (each effect was observed in 1/6 rats at 800 mg/kg-day and 1/6 rats at 1200 mg/kg-day). Treatment had no effect on total implantations, litter size, fetal loss, or the incidence of external malformations. Mean fetal weights were reduced by 13% and 19% in the 800- and 1200-mg/kg-day groups, respectively. For maternal toxicity, NOAEL and LOAEL values of 400 and 800 mg/kg-day, respectively, were identified for reduced body weight, reduced food consumption, and clinical signs. For fetal toxicity, NOAEL and LOAEL values of 400 and 800 mg/kg-day, respectively, are identified for reduced fetal weight.

In the main developmental study, groups of 25 mated female Charles River CD rats were administered 2-nitroaniline (purity not reported) in corn oil at doses of 0, 100, 300, or 600 mg/kg-day on Days 6−15 of gestation (Monsanto Co., 1985). Dams were examined twice daily for mortality and for clinical signs on GD 0, GD 6−20, and GD 21; body weights and food consumption were determined on GD 0, 6, 10, 13, 16, and 21. The dams were sacrificed on GD 21 for gross pathologic examination; dams were not examined for hematological or histopathological effects. Live fetuses were examined for sex distribution, weight, and external, internal, and skeletal malformations. No mortalities of dams were observed. Yellow-to-orange coloring of urine and stained fur occurred in all treated groups; the study authors considered the coloration to be caused by the test material rather than any abnormal physiological process. Food consumption was reduced by 4% ($p \leq 0.05$), 7% ($p \leq 0.05$), and 13% ($p \leq 0.01$) in the 100, 300, and 600 mg/kg groups, respectively, although maternal body weight gain was only significantly decreased in the 600 mg/kg group (6.5% decrease, $p \leq 0.05$).

Signs of maternal toxicity observed in rats treated with 300 mg/kg-day (but not in controls or rats treated with 100 mg/kg-day) included piloerection (2/25 [$p > 0.05$] and 5/25 [$p = 0.05$] rats in the 300 and 600 mg/kg groups, respectively) and pale or cold extremities (1/25 rats in each of the 300- and 600-mg/kg groups). Maternal body weight gain was decreased by 6.5% ($p \leq 0.05$) in the 600 mg/kg group. Pregnancy rates, implantation rates, fetal resorptions, number of litters, fetal viability, mean litter weight, fetal sex distribution, and fetal body weight were comparable to controls in all 2-nitroaniline groups. No fetal external or skeletal malformations were observed. A single fetus in each of two litters from rats receiving 600 mg/kg-day had partial situs inversus (severity nor reported), an abnormality of the heart, which, according to study authors, may have been treatment-related. For maternal toxicity (piloerection, pale or cold extremities), the study authors identified 300 mg/kg-day as a NOAEL and 600 mg/kg-day as a LOAEL. The study authors also identified 300 and 600 mg/kg-day as the NOAEL and LOAEL, respectively, based on the occurrence of situs inversus in fetuses from 2 litters in the 600 mg/kg-day group.

As summarized by OECD/SIDS (2003), Sisti (2001a) evaluated the developmental effects of oral 2-nitroaniline in Sprague-Dawley rats by Sisti (2001a). Mated female rats (unknown sample size) were administered 0, 100, 200, or 400 mg/kg of 2-nitroaniline (purity not reported) in polyethylene glycol 400 by gavage on GDs 0−19 and sacrificed on GD 20. The study followed the OECD (414) Prenatal Developmental Toxicity Study protocol and was conducted under Good Laboratory Practice (GLP) conditions. Endpoints examined in the study are not listed, but, based on reported findings, they include clinical signs, maternal body weight and food consumption, uterine and corrected body weight in dams, total implantations and resorptions, number of corpora lutea, number and sex of viable fetuses, fetal weights, and external malformations. The OECD/SIDS summary does not report group means, incidence data, or results of statistical analysis for any endpoint. OECD/SIDS (2003) does not report data on mortality. Matted fur and piloerection were observed in the 400 mg/kg group, but no clinical signs of toxicity were observed at lower doses or in controls. Cyanosis (as an indicator of methemoglobinemia) was not observed in any 2-nitroaniline group. Slight dose-dependant decreases in body weight (magnitude not reported) were observed in mid-and high-dose dams, but the changes did not reach statistical significance. Body weight gain was significantly reduced in the high-dose dams on GD 6 and 20 in comparison to controls. Uterine weights and corrected body weights in treated dams were comparable to controls. Gross pathological examination of dams did not reveal any treatment-related effects. No treatment-related effects

were observed for total implantations and resorptions, number of viable fetuses, sex ratios, fetal weight, or fetal external malformations. OECD/SIDS (2003) identifies NOAEL and LOAEL values of 200 and 400 mg/kg-day, respectively, for maternal toxicity (apparently based on clinical signs of toxicity in the dams) and a free-standing NOAEL of 400 mg/kg-day for fetal toxicity.

In a reproductive study, 2-nitroaniline (purity not specified) in PEG400 (vehicle) was administered daily by gavage to groups of 12 male (exposure from 4 weeks prior to mating through gestation of females) and 12 female (exposure from 4 weeks prior to mating through Postpartum Day 4) Sprague-Dawley rats (Sisti, 2001b). The rats were treated at doses of 0, 50, 150, or 450 mg/kg body weight, for a total exposure-duration of approximately 9 weeks. The study followed the OECD protocol for Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test (OECD 422, 1996) and was conducted under GLP conditions. Repeated dose toxicity and reproduction/developmental components of the study are presented separately in OECD/SIDS (2003).

Endpoints examined in the repeated dose component of the study are not listed, but, based on reported findings, they include clinical signs, body weight, gross pathology, organ weights (at least in males; organs not specified), and histopathology (no information reported on number of tissues examined). The OECD/SIDS summary does not report group means or incidence data for any endpoint. Signs of clinical toxicity observed immediately after dose administration included piloerection, salivation, and matted fur, although dose-response information is not reported. Matted fur was also observed in high-dose male and female rats during weekly observation sessions. Cyanosis, an indicator of methemoglobinemia, was not observed on examination of adult rats. Compared to controls, body weight was significantly reduced (5 to 6%) at several times throughout the treatment period in mid- and high-dose males and females, at study termination in high-dose males (6%), and on GD 20 and Postpartum Day 4 in high-dose females. Body weight gain in the high-dose dams is reported to be 15% lower than controls on GD 20; no information on magnitude of body weight change at other time points is reported. Gross pathology and histopathological examinations (organs not specified) did not reveal any treatment-related effects.

Endpoints examined for the reproductive/developmental component of the study are not listed, but, based on reported findings, they include reproductive parameters for all adults, litter viability for females, and weight and gross pathology for pups. The OCED/SIDS (2003) summary reported limited information on group means, magnitude of effect, incidence data, or statistical significance. Macroscopic and microscopic observations did not reveal any treatment-related effects on the "spermatogenic cycle" (no additional information reported). Control and treated females showed persistent corpora lutea which was considered to be a physiological condition during lactations. Copulatory and fertility indices, precoital intervals, number of implantations, and prebirth losses were unaffected by treatment with 2-nitroaniline. In litters from high-dose dams with reduced body weight gain on GD 20 or weight loss on Postpartum Day 4, there was a significant increase in the incidence of pup mortality (especially male pups) between birth and Postnatal Day 2; litter size and litter weight were statistically significantly reduced on Day 4 postpartum in the high-dose group when compared to controls, but detailed data are not reported in the OECD/SIDS summary. Necropsy of decedents was unremarkable. Necropsy conducted on remaining pups on Postpartum Day 4 did not reveal differences between control and 2-nitroaniline treatment groups, with the exception of 2 pups

each in the mid- and high-dose groups with abnormal size and color of the median lobe(s) of the liver; additional details are not reported. No information on fetal malformations is reported. Although, OECD/SIDS (2003) identify a NOAEL of 50 mg/kg-day and LOAEL of 150 mg/kg-day for general toxicity in parental animals (apparently based on decreased body weight during treatment) and offspring (apparently based on gross pathological findings in the liver), a body weight changes by 5−6% is usually not considered biologically significant. In addition, the sporadically occurred incidences of abnormal liver pathology in the mid- and high-dose groups without statistical significance was not necessarily treatment related. Based on these considerations, the high dose of 450 mg/kg-day, which caused a 15% reduction in dam body weight gain and induced clinical signs (piloerection, salivation, and matted fur), is considered a LOAEL for maternal toxicity, and the mid-dose of 150 mg/kg-day is the NOAEL. Based on the increased pup mortality and reduced litter weight in the high-dose group, the reproductive NOAEL is 150 mg/kg-day and LOAEL is 450 mg/kg-day.

Inhalation Exposure

Bio/Dynamics (1983a,b) conducted two 4-week inhalation studies in Sprague-Dawley rats. Groups of 10 male and 10 female rats were exposed (whole body) to measured concentrations of 0, 10, 27.5 or 73 mg/m^3 of a vapor/aerosol mix of 2-nitroaniline (purity of 99.6%) 6 hours/day, 5 days/week, for 4 weeks (Bio/Dynamics, 1983a). The test atmospheres for control and all treatment groups contained 2000 mg/m^3 of cellosolve (2-ethoxyethanol); no air-only control group is included. The count median diameter (CMD) of aerosols and geometric standard deviations (GSD) averaged 0.71 ± 1.47, 0.67 ± 1.65, 0.75 ± 1.42 and 0.79 ± 1.5 μm for the 0, 10, 27.5, and 73 mg/m^3 groups, respectively, indicating the presence of a respirable aerosol in all groups (including controls). The corresponding estimated mass median aerodynamic diameters[1] (MMAD) were 1.33 ± 1.47, 1.71 ± 1.65, 1.30 ± 1.42, and 1.55 ± 1.5 μm, respectively. Animals were evaluated for mortality (daily), clinical signs of toxicity (daily), and body weights (weekly). Blood methemoglobin levels were evaluated after 2 and 4 weeks, and complete hematology and clinical chemistry evaluations were performed after 4 weeks of treatment. Ophthalmologic examination was performed before initiation of treatment and at study termination. At study termination, gross pathological examination was performed and selected organ weight (gonads, heart, kidneys, liver, lungs, pituitary, spleen, and brain) were recorded for all groups, with complete histopathological examinations (~32 tissues, including nasal turbinates, lung, mainstream bronchi and peribronchial lymph nodes) for control and 73 mg/m^3 groups. Only the testes and epididymides were examined microscopically in rats exposed to 10 or 27.5 mg/m^3.

Inhalation of 2-nitroaniline for 4 weeks had no significant effect on survival, with no chemical-related mortalities occurring during the study; one female in the 10 mg/m^3 group and one male in the 73 mg/m^3 group died accidentally during the interim blood collection (Bio/Dynamics, 1983a). Mean body weights in the 2-nitroaniline groups were comparable to controls throughout the study. Yellow discoloration of the fur was observed in all rats exposed to 2-nitroaniline; the study authors attributed this coloration to physical deposition of the test material. Other treatment-related clinical signs, including lacrimation and red or dried red nasal discharge, are consistent with irritant effects of the eyes and upper respiratory tract, but the rats

[1] MMAD = $\rho^{0.5}$CMAD × exp [3 × (ln σ_g)^2] (U.S. EPA, 1994b); σ_g = GSD, ρ = particle density in g/cm^3

did not exhibit consistent dose- or duration-dependent relationships with 2-nitoranaline (Table 2). These weekly physical observations are reported only as a summary of males and females combined.

Table 2. Clinical Signs Observed in Male and Female Rats Exposed to Inhaled 2-Nitroaniline for 4 Weeks[a]

Observation[c]	Exposure Group (mg/m^3)[b]			
	0	10 (HEC[d] = 0.55)	27.5 (HEC = 1.5)	73 (HEC = 3.9)
Week 1				
Lacrimation	0[e]	0	0	0
Mucoid nasal discharge	0	0	1	0
Red nasal discharge	0	0	1	0
Dried red nasal discharge	0	0	1	2
Week 2				
Lacrimation	0	0	0	2
Mucoid nasal discharge	0	0	1	0
Red nasal discharge	0	0	0	0
Dried red nasal discharge	3	2	8	4
Week 3				
Lacrimation	0	0	0	1
Mucoid nasal discharge	0	0	0	2
Red nasal discharge	0	0	0	2
Dried red nasal discharge	0	0	3	5
Week 4				
Lacrimation	1	0	1	4
Mucoid nasal discharge	0	1	0	1
Red nasal discharge	0	0	1	1
Dried red nasal discharge	1	7	1	1

[a]Bio/Dynamics, 1983a
[b]Data were reported as combined incidence for males and females
[c]20 rats in 0 and 27.5 mg/m^3 groups; 19 rats in 10 and 73 mg/m^3 groups (1 rat/group died during the 2-week blood collection)
[d]HEC: human equivalent concentration. The detailed calculation method for the tissue specific HEC is shown in the section of derivation of provisional subchronic and chronic inhalation p-RfC. The HEC here is based on an RDDR for the extrathoracic region.
[e]Number of rats with observation

The effects of 2-nitroaniline treatment on hematological parameters include significant ($p \leq 0.01$) reductions in leukocyte counts in males and in hemoglobin content and erythrocyte count in females exposed to 73 mg/m^3 (Table 3) (Bio/Dynamics, 1983a). No effects were observed for Hct, MCH, mean cell hemoglobin concentration (MCHC), or mean cell volume (MCV). In addition, aberrant erythrocyte morphology (mild-to-moderate anisocytosis, poikilocytosis, and polychromia) was observed in animals of both sexes at 73 mg/m^3 (incidence data not reported). Mean methemoglobin levels were not affected by treatment. The study authors considered the minimal hematological changes to be suggestive of a treatment relationship. 2-Nitroaniline had no effect on clinical chemistry parameters.

Table 3. Selected Hematology Parameters in Male and Female Sprague-Dawley Rats Exposed to Inhaled 2-Nitroaniline for 4 Weeks[a]

Parameter	Exposure Group (mg/m^3)			
	0	10 (HEC[b] = 6.0)	27.5 (HEC = 16)	73 (HEC = 45)
Males[c]				
Hgb (g/dL)	14.9 ± 1.0[d]	15.0 ± 0.6	15.4 ± 1.7	14.7 ± 0.9
2-Week MetHgb (% of total Hgb)	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.4
4-Week MetHgb (% of total Hgb)	0.6 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	0.8 ± 0.2
RBC count (10^6/µL)	7.22 ± 1.42	7.28 ± 0.48	7.35 ± 0.29	7.11 ± 0.35
WBC count (10^3/µL)	10.3 ± 3.0	9.0 ± 2.0	8.1 ± 1.3	6.8 ± 1.6[e]
Females[e]				
Hgb (g/dL)	14.9 ± 0.9	15.0 ± 0.7	15.4 ± 1.5	14.0 ± 0.9[e]
2-Week MetHgb (% of total Hgb)	0.6 ± 0.3	0.7 ± 0.3	0.7 ± 0.2	0.9 ± 0.5
4-Week MetHgb (% of total Hgb)	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.2
RBC count (10^6/µL)	7.00 ± 0.42	6.93 ± 0.42	7.12 ± 0.21	6.41 ± 0.32[e]
WBC count (10^3/µL)	8.1 ± 3.0	6.9 ± 1.9	8.2 ± 4.4	6.2 ± 2.1

[a]Bio/Dynamics, 1983a
[b]The HEC here is based on an RDDR for the extrarespiratory region.
[c]Number of rats per group: control = 10; 10 mg/m^3 = 10; 27.5 mg/m^3 = 10; 73 mg/m^3 = 9
[d]Mean ± SD
[e]Significantly different from control ($p \leq 0.01$)
[f]Number of rats per group: control = 10; 10 mg/m^3 = 9; 27.5 mg/m^3 = 10; 73 mg/m^3 = 10

No treatment-related findings were observed on ophthalmologic examination. No treatment-related findings were observed on gross or microscopic examination of the lung, mainstream bronchi or nasal turbinates. Relative liver weight was increased by 10% ($p \leq 0.05$) in females treated with 73 mg/m^3, but no treatment-related hepatic histopathology was observed (Bio/Dynamics 1983a). The gross examination of males revealed an increased incidence of small testes (bilateral) in the 73 mg/m^3 group (incidence 0/10, 1/10, 0/10, and 9/9 in the 0, 10, 27.5 and 73 mg/m^3 groups, respectively), with small seminal vesicles in 2/10 high exposure males and decreased secretory product in one of these animals. Absolute and relative testicular weights are significantly ($p \leq 0.01$) reduced, by 32.4% and 30% respectively, in the 73 mg/m^3

group. Microscopic examination revealed mild-to-moderate bilateral degeneration of the testicular germinal epithelium in all high-exposure males. In males exposed to 10 mg/m^3, bilateral testicular degeneration was observed in 2/10 animals (one minimal and one marked severity) and minimal unilateral testicular degeneration was observed in 3/10 animals, although absolute and relative testes weights were comparable to controls. No testicular effects were observed in the control or 27.5 mg/m^3 groups. Based on testicular degeneration in male rats, a LOAEL of 10 mg/m^3 (HEC = 6.0 mg/m^3) is identified for 4-week inhalation exposure to an aerosol/vapor mixture of 2-nitroaniline and 2000 mg/m^3 of cellosolve.

Due to uncertainty regarding the role of cellosolve in inducing the testicular effects in the previously described inhalation study, Bio/Dynamics (1983b) performed a second inhalation study without the use of cellosolve. Groups of 10 male Sprague-Dawley rats were exposed (whole body) to 2-nitroaniline in air at measured concentrations of 9.8 or 92 mg/m^3 for 6 hours/day, 5 days/week, for 4 weeks. The test atmospheres were generated by passing heated nitrogen over the heated test material. A control group of 10 male rats was exposed to air containing additional nitrogen, approximating the quantity used to generate the high-group test atmosphere. The count median aerodynamic diameter (CMAD) ± GSD for the control were 0.53 ± 1.9 μm and low-exposure 0.55 ± 2.1 μm. The estimated mass median aerodynamic diameter[2] (MMAD) corresponding to these exposures were 1.8 μm and 2.9 μm, respectively. For the high-exposure atmosphere, the study authors reported particle size in terms of MMAD ± GSD to be 3.6 ± 2.7 μm. Rats were examined once daily for signs of toxicity, twice daily for mortality, and weekly for body weight. At termination, blood was collected for hematology analysis and all rats were given a complete gross necropsy; the brain and testes (with epididymides) of all rats were weighed, and the testes with epididymides were examined for histopathology in control and high-exposure rats.

The study authors considered that the control and low-concentration treatment atmospheres were not significantly different from each other or from comparative room air samples (CMAD of 0.55−0.57 ± 1.9 μm). Based on this consideration, the study authors concluded that 2-nitroaniline existed mainly as vapor in the low-level treatment atmosphere. This conclusion was apparently based on a comparison of aerosol sizes in the unit of CMAD. However, when we expressed the exposure aerosol size as a common aerosol size of MMAD for all three experimental groups, we noted a clear concentration-related increase in the control (MMAD of 1.8 μm), low concentration (MMAD of 2.9 μm), and high concentration (MMAD of 3.6 μm). Based on this concentration related increase in aerosol size and chemical's high boiling temperature of 284^{o}C (>250^{o}C), this chemical should be considered nonvolatile (EU, 1004). Therefore, the inhalation exposure was more likely presented in the form of aerosol instead of vapor. Thus, all the dosimetric adjustments for the HEC are based on a regional deposited dose ratio (RDDR) (U.S. EPA, 1994b).

No mortalities were observed in any treatment group throughout the study, and mean bodies weights in the 2-nitroaniline treatment groups were comparable to controls. Yellow discoloration of the fur was observed in all rats exposed to 2-nitroaniline; the study authors attributed this coloration to physical deposition of the test material. Other clinical observations, such as lacrimation and nasal discharge, were indicative of ocular and upper respiratory irritation (Table 4). The dose-dependent increased incidence of lacrimation was observed from Exposure

[2] MMAD=CMAD × exp [3 × (ln $⑨_g$)^2] (U.S. EPA, 1994b); $⑨_g$ = GSD

Week 1. The incidence of nasal discharge generally increased in a dose- and duration-dependent fashion. Severity of irritation was not graded and histopathologic examination of nasal tissue was not performed.

Table 4. Incidence of Selected Clinical Signs Observed in Male Rats Exposed (Whole Body) to Inhaled 2-Nitroaniline for 4 Weeks[a]

Observation[b]	Exposure Group (mg/m^3)		
	0	**9.8 (HEC[c] = 0.46)**	**92 (HEC = 3.5)**
Week 1			
Lacrimation	5[d]	5	7
Mucoid nasal discharge	0	0	5
Red nasal discharge	0	1	0
Dried red nasal discharge	2	1	2
Week 2			
Lacrimation	3	5	10
Mucoid nasal discharge	0	2	2
Red nasal discharge	0	1	3
Dried red nasal discharge	2	0	5
Week 3			
Lacrimation	6	10	10
Mucoid nasal discharge	2	5	5
Red nasal discharge	1	1	0
Dried red nasal discharge	0	0	0
Week 4			
Lacrimation	5	10	9
Mucoid nasal discharge	2	3	3
Red nasal discharge	0	0	0
Dried red nasal discharge	1	2	6

[a]Bio/Dynamics, 1983b
[b]10 rats/treatment group
[c]HEC here was based on an RDDR for the extrathoracic region.
[d]Number of rats with observation

Increased blood methemoglobin and slightly increased hematocrit were observed in rats exposed to 92 mg/m^3 (Table 5), although total Hgb, RBC count, reticulocyte count, and WBC count were comparable to controls in both exposure groups. WBC differential count showed a significant decrease (67%) in segmented neutrophils ($p \leq 0.05$). No treatment-related findings were observed upon gross pathological examination.

Table 5. Selected Hematology Parameters in Male Sprague-Dawley Rats Exposed to Inhaled 2-Nitroaniline for 4 Weeks[a]

Parameter[b]	Exposure Group (mg/m^3)		
	0	9.8 (HEC[c] = 5.0)	92 (HEC = 42)
Hgb (g/dL)	17.2 ± 0.7[d]	16.8 ± 0.4	17.2 ± 0.5
MetHgb (% of total Hgb)	0.5 ± 0.2	0.4 ± 0.21	0.7 ± 0.2[e]
Hct (%)	48 ± 2	48 ± 1	50 ± 2[e]

[a]Bio/Dynamics, 1983b
[b]10 rats/group
[c]The HEC here was based on an RDDR for the extrarespiratory region.
[d]Mean ± SD
[e]Significantly different from control ($p \leq 0.05$)

Absolute and relative weights of brain and testes in rats exposed to 2-nitroaniline were comparable to controls. Histological examination of control and high-exposure rats revealed testicular degeneration in 1/10 rats in each group and sperm granuloma in the epididymides of another high-exposure male; however, the study authors did not consider the effects to be treatment-related.

Compared to the effective concentration 98 mg/m^3 (HEC = 42 mg/m^3) for increased methemoglobin and hematocrit in this study, a LOAEL of 9.8 mg/m^3 (HEC = 0.46 mg/m^3) for minor local irritant effects (lacrimation and nasal discharge) is identified to be the most sensitive effective concentration in the study.

Studies evaluating the effects of inhaled 2-nitroaniline on reproduction and fetal development are not identified.

Other Studies

Studies Comparing 2-, 3-, and 4-Nitroaniline

Methemoglobinemia has been identified as a primary adverse effect of subchronic and chronic oral exposure to other aniline and substituted aniline compounds, including 3- and 4-nitroaniline (NTP, 1993a; OECD/SIDS, 1994); however, data are inconsistent regarding the relative potency of the nitroaniline isomers to induce methemoglobinemia, possibly reflecting species/strain differences. Acute (presumably oral) treatment of rats with 4-nitroaniline, mice with 2-nitroaniline, and guinea pigs with either isomer-induced effects on the erythrocytes, including the development of Heinz bodies; animal strains are not reported (Moskalenko, 1966). In Sprague-Dawley rats administered 150 mg/kg of nitroaniline isomer by gavage, methemoglobin levels 6 hours later were not affected by 2-nitroaniline (0.9% vs. 1% in controls), whereas they were elevated to 10.5 and 11.6% by exposure to 3- and 4-nitroaniline, respectively (SOCMA, 1984). In male Wistar rats injected i.p. with 100 μM nitroaniline isomers, methemoglobin levels 5 hours after injection were significantly elevated compared to controls: 14.2, 12.9, and 11.0% for 2-, 3-, and 4-nitroaniline, respectively (Watanabe et al., 1976). Eastman Kodak Co. (1965, 1969) reported that dermal or inhalation exposure to 2- or

4-nitroaniline induced cyanosis due to methemoglobin formation (no further details are provided). Results of available studies show that 2-nitoaniline induces methemoglobinemia, but data are insufficient to identify the relative potencies of the nitroaniline isomers.

In vitro studies demonstrated significant methemoglobin-inducing activity of nitroaniline isomers. 2-Nitroaniline was less potent than 4-nitroaniline but more potent than 3-nitroaniline in inducing methemoglobin formation in freshly drawn sheep erythrocytes (French et al., 1995). Methemoglobin levels about 4 times higher than the control were produced by treatment with 0.05 mM 2-nitroaniline, 0.005 mM 4-nitroaniline, or 0.25 mM 3-nitroaniline. All three isomers required the presence of an NADP bioactivation system to induce methemoglobin. The methemoglobin-forming potency of 2-nitroaniline in sheep erythrocytes was calculated to be about one-third lower than 4-nitroaniline but 13 times higher than aniline. In another study, incubation of 0.5 moles of 2- or 4-nitroaniline with 0.1 mole of hemoglobin from Wistar rats resulted in the same percentage being converted to methemoglobin (5.7%) after 5 hours (Watanabe et al., 1976).

Acute LD_{50} data (Table 6) suggest that 2-nitroaniline may be slightly less toxic than 4-nitroaniline, but similar signs of toxicity have been reported for the two isomers. In rats treated orally with 2-nitroaniline, death was preceded by reduced appetite and activity, increasing weakness and collapse; gross examination of decedents revealed hemorrhagic lungs, hyperemia of the liver, gastrointestinal inflammation, and a deep yellow color of the tissues and urine (Younger Labs, Inc., 1983a). In rats treated with 4-nitroaniline, death was preceded by tremors and convulsions, but necropsy findings were similar to those in rats treated with 2-nitroaniline (Younger Labs, Inc., 1983b). Death was preceded by spasms in rats given lethal oral doses of either isomer (Moskalenko, 1966). Rats acutely exposed by inhalation to either isomer showed slight corneal opacity within 24-hours (du Pont, 1982a,b).

Genotoxicity Studies

The genotoxicity of 2-nitroaniline has been investigated in bacterial systems in several studies. Reverse mutation assays in *Salmonella typhimurium* have yielded conflicting results in the presence of metabolic activators (Shimizu and Yano, 1986; Le et al., 1985; Thompson et al., 1983; Chiu et al., 1978; Garner and Nutman, 1977), although no mutagenic activity has been observed in the absence of metabolic activation (Shimizu and Yano, 1986; Thompson et al., 1983; Garner and Nutman, 1977). One study in *Escherichia coli* reported negative results for reverse mutation with metabolic activation (data not reported without activation) (Thompson et al., 1983). No evidence of genotoxicity of 2-nitroaniline has been observed in mammalian cells, although data are limited. In primary cultured hepatocytes, 2-nitroaniline did not induce unscheduled DNA synthesis (Yoshimi et al., 1988; Thompson et al., 1983). In vivo, 2-nitroaniline induced a small increase in micronuclei in the bone marrow of male—but not female—mice (Blakey et al., 1994). Overall, the results of available studies do not provide convincing evidence that 2-nitroaniline has genotoxic activity.

Table 6. Acute Oral LD_{50} Values of 2- And 4-Nitroaniline

Species	Nitroaniline Isomer LD_{50} (mg/kg)		Reference
	2-Nitroaniline	4-Nitroaniline	
Rat	2050	1400	Younger Labs, Inc., 1983a,b
Rat	3560	3250	Vernot et al., 1977
Rat	3520	1410	Vasilenko et al., 1974
Rat	NR	1500	Moskalenko, 1966
Rat	400−3200	400−3200	Eastman Kodak Co., 1965, 1969
Mouse	1290	810	Vernot et al., 1977
Mouse	1246	NR	Moskalenko, 1966
Guinea pig	2350	450	Moskalenko, 1966

NR: not reported

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfDs FOR 2-NITROANILINE

The database for 2-nitroaniline includes a short-term 14-day study (Komsta et al., 1989), three developmental studies (Monsanto Co., 1984, 1985; Sisti 2001a), and a 9-week reproductive study (Sisti, 2001b). The 14-day oral toxicity study in rats (Komsta et al., 1989) examined an array of appropriate endpoints (with an exception of methemoglobin) and found no effects of 2-nitroaniline exposure at any dose tested (a freestanding NOAEL of 100 mg/kg-day). This study is not suitable for derivation of provisional RfDs for 2-nitroaniline due to the short exposure duration.

The developmental studies in rats by Monsanto Co. (1984, 1985) showed overt effects (clinical signs, decreased body weight) in the dams at doses of 600−800 mg/kg-day during gestation, but they did not include investigation of more sensitive systemic endpoints, hematology. These studies did not evaluate hematological parameters or conduct gross or histopathological examinations of organs typically affected by the development of methemoglobinemia (e.g., spleen, liver, and bone marrow). Additionally, decreased fetal weight (Monsanto Co., 1984) and a marginal effect on internal morphological abnormality (situs inversus of the heart) (Monsanto Co., 1985) were also observed at doses of 600−800 mg/kg-day. Another developmental study conducted by Sisti (2001a), with a longer exposure duration of GD 0−19, found similar responses: clinical signs and slight decreases in body weight in the dams exposed to 400 mg/kg-day of 2-nitroaniline, and no effects on fetal development.

The 9-week reproductive study (Sisti, 2001b) provides a little more information about the systemic toxicity due to exposure to 2-nitroaniline. In addition to reproductive parameters, this study also examines gross pathology, organ weight, and histopathology. The study indicates a NOAEL of 150 mg/kg-day based on reduced body weight gain and clinical signs in dams and increased mortality and reduced litter weight at the high dose of 450 mg/kg-day.

Blood has been established as a sensitive target for oral exposure to nitroanilines (NTP, 1993a); specific effects include the formation of methemoglobin and subsequent development of hemolytic anemia and compensatory erythropoiesis. However, the database on 2-nitroaniline does not provide information on this key parameter from any of these studies available. In addition, the database also lacks a comprehensive subchronic study. As the result, no provisional subchronic or chronic RfD is estimated. Nevertheless, the Appendix of this document contains screening subchronic and chronic RfDs that may be useful in certain instances. The reason for developing only screening values is the uncertainty in determining the most sensitive response and greater composite uncertainty values. Please see Appendix A for details.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfCs FOR 2-NITROANILINE

Inhalation data on 2-nitroaniline come from two 4-week inhalation studies in rats (Bio/Dynamics, 1983a,b), one of which appears to have been compromised by the use of 2000 mg/m^3 cellosolve (2-ethoxyethanol) as a vehicle (Bio/Dynamics, 1983a). Irritant effects, as well as toxicity to the testes, decreased RBC count, and increased liver weight were observed in this study, but similar findings have also been reported in toxicity assays on cellosolve alone (NTP, 1993b; RTECS, 2000). Therefore, interpretation of these data was confounded by use of cellosolve. The 4-week study conducted without cellosolve as a vehicle identified the eyes, upper respiratory tract, and blood as targets for inhaled 2-nitroaniline (Bio/Dynamics, 1983b). Signs of ocular (lacrimation) and nasal (red or dried red discharge) irritation occurred in rats exposed to 2-nitroaniline concentrations of 9.8 mg/m^3 (HEC = 0.46 mg/m^3) and 92 mg/m^3 (HEC = 3.5 mg/m^3), and hematological effects (increased methemoglobin and Hct; decreased leukocyte counts) occurred in male rats exposed to 92 mg/m^3 (HEC = 42 mg/m^3). As summarized in Table 4, at 2-nitroaniline concentrations of 9.8 and 92 mg/m^3, 100% of animals showed signs of ocular irritation while 50% of animals in the control group also showed the similar responses within 3 weeks of exposure; therefore, such data casted some uncertainty in identified ocular irritation. Considering the same response only started at 73 mg/m^3 (4/19 rats vs. 1/20 in the control group) in the other inhalation study (Bio/Dynamics, 1983a), the lower concentration of 9.8 mg/m^3 (HEC = 0.46 mg/m^3) in Bio/Dynamics (1983b) study, was considered a conservative LOAEL for the ocular irritation response in rats. A dose-dependent increase in nasal irritation was also observed in Bio/Dynamics (1983b) study, and a marginal increase (3/10 vs. 2/10 in the control for the mucoid nasal discharge and 2/10 vs. 1/10 in the control for the dried red nasal discharge) started at the low concentration level (9.8 mg/m^3). Thus, this concentration (9.8 mg/m^3 or a HEC of 0.46 mg/m^3) is considered the LOAEL for the overall irritation responses. Methemoglobinemia occurred only in rats exposed to the high concentration of 2-nitroaniline (HEC = 42 mg/m^3), which is approximately 10-fold greater than concentrations producing ocular and nasal irritation. Therefore, we selected ocular/nasal irritation (Bio/Dynamics, 1983b), with a LOAEL of 9.8 mg/m^3 (HEC = 0.46 mg/m^3), as the critical effect for derivation of the subchronic and chronic p-RfCs. Benchmark dose (BMD) modeling could not be conducted for the irritant effects because the detailed incidence data for individual animals are not available for upper airway irritation, and there is a plateau response for the lacrimation.

To calculate the subchronic p-RfC for 2-nitroaniline, we first adjusted the LOAEL of 9.8 mg/m^3 in male rats (Bio/Dynamics, 1983b) for continuous exposure ($LOAEL_{ADJ}$), as recommended by U.S. EPA (1994b). Although irritant effects are typically more closely associated with concentration than duration of exposure, there is evidence for 2-nitroaniline that the incidence of respiratory irritation increased with duration of exposure over the 4 weeks of the study (Bio/Dynamics, 1983b). Because duration of exposure appears to contribute to the observed effect, the duration adjustment was performed. The $LOAEL_{ADJ}$ is calculated as follows (U.S. EPA, 1994b):

$$\begin{aligned} LOAEL_{ADJ} &= (LOAEL)\ (\#\ hours/24\ hours)\ (\#\ days/7\ days) \\ &= (9.8\ mg/m^3)\ (6\ hours/24\ hours)\ (5\ days/7\ days) \\ &= 1.75\ mg/m^3 \end{aligned}$$

The HEC ($LOAEL_{HEC}$) based on the $LOAEL_{ADJ}$ was calculated for a respiratory effect (nasal irritation) of aerosol in the extrathoracic region by multiplying the $LOAEL_{ADJ}$ by the rat:human extrathoracic regional deposited dose ratio ($RDDR_{ET}$). The HEC for hematological effect of aerosol in the extrarespiratory region was calculated by multiplying the $LOAEL_{ADJ}$ by the rat:human extrarespiratory regional deposited dose ratio ($RDDR_{ER}$). The $RDDR_{ET}$ or $RDDR_{ER}$ was calculated from RDDR software provided with RfC guideline (U.S. EPA, 1994b). Table 7 summarizes all the physiological and aerosol parameters used in the calculation and the resulting HECs.

Table 7. Summary of Parameters Used in Calculation of Human Equivalent Concentration Calculations

C	C_{adj}	Average body weight (g)[a]	CMAD (µm)	Sigma g (GSD) (µm)	MMAD (µm)	$RDDR_{ET}$	HEC_{ET} (mg/m^3)	$RDDR_{ER}$	HEC_{ER} (mg/m^3)
0	0	322	0.53	1.9	1.8		0		
9.8 mg/m^3	1.75	328	0.55	2.1	2.8	0.262	**0.459**	2.87	5.02
92 mg/m^3	16.4	323	N/A	2.7	3.6	0.215	3.53	2.56	42.0

[a]Average body weight was calculated based on reported body weight data shown in the Appendix E-1 in the Bio/Dynamics (1983b) report.

C_{ADJ} = duration adjusted exposure concentration (mg/m^3)
GSD = geometric standard deviation (also called sigma g in RDDR software)
MMAD = CMAD × exp [3 × (ln ⑨$_g$)^2] (U.S. EPA, 1994b)
⑨$_g$ = GSD
$RDDR_{ET}$ = regional deposited dose ratio for extra thoracic region (estimated from RDDR software)
$RDDR_{ER}$ = regional deposited dose ratio for extra respiratory region (estimated from RDDR software)
HEC = human equivalent concentration (mg/m^3) calculated as following:

The $LOAEL_{HEC}$ of 0.459 mg/m^3 was calculated as follows:

$$\begin{aligned} LOAEL_{HEC} &= LOAEL_{ADJ} \times RDDR_{ET} \\ &= 1.75 \text{ mg/m}^3 \times 0.262 \\ &= 0.459 \text{ mg/m}^3 \end{aligned}$$

The **subchronic p-RfC of 0.0004 mg/m^3 or 4 × 10^{-4} mg/m^3** for 2-nitroaniline, based on the $LOAEL_{HEC}$ of 0.459 mg/m^3 in male rats (Bio/Dynamics, 1983b), is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfC} &= LOAEL_{HEC} \div UF \\ &= 0.459 \text{ mg/m}^3 \div 1000 \\ &= \textbf{0.0004 mg/m}^3 \textbf{ or } \textbf{4 × 10}^{-4} \textbf{ mg/m}^3 \end{aligned}$$

The uncertainty factor of 1000 is composed of the following:

- A default 10-fold UF for intraspecies differences accounts for potentially susceptible individuals in the absence of information on the variability of response in humans. Individuals with preexisting respiratory disorders, such as asthma or emphysema, may be more susceptible to inhaled 2-nitroaniline.
- A partial 3-fold UF ($10^{0.5}$) for interspecies extrapolation accounts for potential toxicodynamic differences between rats and humans. Converting the rat data to HECs by the dosimetric adjustment accounts for toxicokinetic differences between rats and humans; thus, it is not necessary to use the default UF of 10 for interspecies extrapolation.
- A partial 3-fold UF ($10^{0.5}$) accounts for use of a LOAEL. The LOAEL was derived based on minor, local irritant effects in the Bio/dynamics (1983b) that had a high percentage of background response (50%). However, in another similar rat study with no background irritation response (Bio/dynamics, 1983a), the similar response only started at a concentration level about 3-fold higher (27 mg/m^3). Therefore, the LOAEL of 9.8 mg/m^3 (HEC = 0.459 mg/m^3) represents a minimally biologically significant response level. As the result, only a partial UF is used.
- A 10-fold UF is included for database insufficiencies. The database lacks reproductive and developmental toxicity studies by the inhalation route although oral studies suggested that developmental and reproductive responses are not sensitive endpoints for 2-nitroaniline systemic toxicity. Thus, a partial UF is used to account for no direct developmental or reproductive studies by inhalation route of exposure.

This subchronic p-RfC of 0.0004 mg/m^3 is 50-fold lower than the subchronic p-RfC of 0.02 mg/m^3 for 4-nitroaniline. As summarized before, methemoglobinemia has been identified as a primary adverse effect of subchronic and chronic oral exposure to anilines; however, in vivo and in vitro data are inconsistent regarding the relative potency of the nitroaniline isomers to induce methemoglobinemia. The subchronic p-RfC for 2-nitroaniline is based on the $LOAEL_{HEC}$ of 0.459 mg/m^3 for a respiratory effect (nasal irritation) of aerosol in a 4-week study rather than the $NOAEL_{[HEC]}$ of 5.02 mg/m^3 for methemoglobinemia. The same study identifies a $LOAEL_{HEC}$ of 42 mg/m^3 (Table 7) based on methemoglobinemia; it is within 2-fold range of a $LOAEL_{HEC}$ of 23 mg/m^3 based on the same endpoint for 4-nitroaniline in a 2-week rat study (DuPont Co., 1994). Because DuPont Co. (1994) did not report any irritation response after 4-nitroaniline inhalation exposure, the nasal irritation response could be a unique response to 2-nitroaniline inhalation exposure. As a result, the POD for 2-nitroaniline RfC is based on this more sensitive response; therefore, it results in a much lower subchronic p-RfC than the p-RfC for 4-nitroaniline.

Confidence in the critical study is low to medium. Although the critical study had several shortcomings, such as only including two treated groups, encompassing a relatively short duration, including only males, limiting the histopathologic examination to tissues of the testes plus epididymides, another similar inhalation study provides some supportive evidence on the response observed. Confidence in the database is medium due to lack of developmental and reproductive studies by inhalation route. Although no developmental or reproductive toxicity studies by the inhalation route are available, studies by the oral route suggest that 2-nitroaniline does not adversely affect reproduction or fetal development in the absence of maternal toxicity. The overall confidence in the subchronic p-RfC is low to medium.

Derivation of a chronic p-RfC is precluded because of the requirement for a composite UF of 10,000. A screening chronic RfC is provided in Appendix A.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 2-NITROANILINE

Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to 2-nitroaniline in humans or animals are not identified in the available literature. The limited genotoxicity data were primarily negative. Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), "*Inadequate Information is Available to Assess Carcinogenic potential.*"

Quantitative Estimates of Carcinogenic Risk

The lack of suitable data precludes the derivation of quantitative estimates of cancer risk for 2-nitroaniline.

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APPENDIX A. DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL SCREENING RfDs AND A CHRONIC INHALATION SCREENING RfC FOR 2-NITROANILINE

Screening Subchronic and Chronic RfDs

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for 2-nitroaniline. 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. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

The database for 2-nitroaniline includes a short-term 14-day study (Komsta et al., 1989), three developmental studies (Monsanto Co., 1984, 1985; Sisti, 2001), and a 9-week reproductive study (Sisti, 2001b). All these studies were conducted in various strains of rats. The 14-day oral toxicity study in SD rats (Komsta et al., 1989) examined an array of appropriate endpoints including hematology parameters and tissue histopathology. However, this study provides no information on methemoglobin, which might be a more sensitive indicator of toxicity for this compound. This short-term study found no toxicity effects at 2-nitroaniline doses as high as 100 mg/kg-day, suggesting a free-standing NOAEL of 100 mg/kg-day.

The developmental studies in rats (Monsanto Co., 1984, 1985) with an exposure duration of GD 6−15 showed overt effects (clinical signs, slightly decreased body weight) in the dams at doses ≥600−800 mg/kg-day during gestation. At these dose levels, treatment caused reduced fetal body weight or low incidences of situs inversus (an abnormality of the heart). No other developmental or maternal toxicity effects were reported. Similar to most of regular developmental studies, the studies on 2-nitroaniline do not include investigation of the effects on hematology, a more sensitive systemic endpoint. Regarding the potential developmental effects, no evidence of adverse fetal development associated with oral exposure to 2-nitroaniline is observed in these studies because the decrease in fetal weight (Monsanto Co., 1984) and the marginal effect on internal morphological abnormality (situs inversus of the heart) (Monsanto Co., 1985) were only observed at the doses producing maternal toxicity. These studies identify the NOAEL of 300 mg/kg-day and LOAEL of 600 mg/kg-day for both maternal and developmental toxicities. Another developmental study conducted by Sisti (2001a), which has a longer exposure duration (GD 0−19), found similar responses (e.g., clinical signs and slight decreases in body weight, in the dams exposed to 400 mg/kg-day of 2-nitroaniline, and no effects on fetal development). This study provides the lowest NOAEL of 200 mg/kg-day among all the developmental studies (Monsanto Co., 1984, 1985; Sisti, 2001a) based on maternal toxicity.

The 9-week reproductive study (Sisti, 2001b) provided a little more information about the systemic toxicity due to exposure to 2-nitroaniline. In addition to reproductive parameters, this study also examines gross pathology, organ weight, and histopathology. Because this study followed the OECD Test Guideline (OECD 422, 1996), it should also include the histopathology of organs including the liver, spleen, and bone marrow. This study suggests a NOAEL of 150 mg/kg-day based on reduced body weight gain and clinical signs, in dams, and increased pup mortality and reduced litter weight at the high dose of 450 mg/kg-day.

The NTP (1993a) established blood as a sensitive target for oral exposure to nitroanilines; specific effects include the formation of methemoglobin and subsequent development of hemolytic anemia and compensatory erythropoiesis. Because the two developmental studies (Monsanto Co., 1985; Sisti, 2001a) did not evaluate hematological parameters or conduct gross or histopathological examinations of organs typically affected by the development of methemoglobinemia (e.g., spleen, liver, and bone marrow), we could not use these studies to derive a screening subchronic RfD. However, both the short-term study and the reproductive study provide some information regarding haematological effects. The haematological and histological examinations in the 14-day study did not show any adverse effects. In addition, the 9-week reproductive study supposedly also examined relevant organs, such as the liver, spleen, and bone marrow, but they reported no adverse effects after the exposure. Even though none of these studies examined the potential sensitive index of 2-nitroaniline, methemoglobin, the negative finding from the relevant organs or tissues provides enough information for derivation of a screening subchronic RfD, although with some uncertainty in finding the most sensitive responses. The NOAEL of 150 mg/kg-day, identified from the 9-week reproductive study, is considered an appropriate POD for derivation of a screening subchronic RfD for its longer duration than the 14-day study, and more comprehensive examination of systemic toxicity.

The **screening subchronic RfD of 0.15 mg/kg-day or 1×10^{-1} mg/kg-day** for 2-nitroaniline, based on the NOAEL of 150 mg/k-day in female rats and pups (Sisti, 2001b), is derived as follows:

$$\begin{aligned} \textbf{Screening Subchronic RfD} &= \text{NOAEL} \div \text{UF} \\ &= 150 \text{ mg/kg-day} \div 1000 \\ &= \textbf{0.15 mg/kg-day or } \mathbf{1 \times 10^{-1}} \textbf{ mg/kg-day} \end{aligned}$$

The UF of 1000 is composed of the following:

- A default 10-fold UF for intraspecies differences accounts for potentially susceptible individuals in the absence of information on the variability of response in humans.
- A default 10-fold UF for interspecies extrapolation accounts for potential toxicokinetic and toxicodynamic differences between rats and humans.
- A default 10-fold UF accounts for database insufficiencies. Although the database includes a 14-day study, two developmental studies, and a reproductive study, all these studies were conducted in rats. The database does not include information regarding the potentially most sensitive response, methemoglobin.

The screening subchronic RfD of 0.15 mg/kg-day is 15-fold higher than the subchronic p-RfD of 0.01 mg/kg-day for 4-nitroaniline. As summarized before, methemoglobinemia has been identified as a primary adverse effect of subchronic and chronic oral exposure to anilines; however, in vivo and in vitro data are inconsistent regarding the relative potency of the nitroaniline isomers to induce methemoglobinemia. The lack of data precludes a direct estimation of subchronic RfD based on other nitroaniline isomers. Because the POD for 2-nitroaniline is based on systemic toxicity, the lack of a POD based on critical hematological response significantly decreases the confidence in the critical study.

Confidence in the critical study is low because methemoglobin is not examined in the critical study. Confidence in the database is medium due to lack of a development study in second animal species, and the absence of a full duration subchronic study. However, the current database does provide study duration up to 9 weeks, and it includes two developmental studies and a reproductive study in rats. The overall confidence in the screening subchronic RfD is low to medium.

Chronic toxicity studies for oral exposure to 2-nitroaniline are not available. Therefore, the screening chronic RfD is based on the NOAEL of 150 mg/kg-day identified from the 9-week reproductive study used for derivation of the screening subchronic RfD. The **screening chronic RfD of 0.015 mg/kg-day or 1×10^{-2} mg/kg-day** for 2-nitroaniline is derived as follows:

Screening Chronic RfD = NOAEL ÷ UF
= 150 mg/kg-day ÷ 10,000
= **0.015 mg/kg-day or 1×10^{-2} mg/kg-day**

The uncertainty factor of 10,000 is composed of the same UF applied for the screening subchronic RfD plus an extra UF for using a NOAEL from a less-than-chronic exposure duration study. A default UF of 10 for less-than-chronic exposure duration (duration of the critical study was only 9 weeks) accounts for the possibility that more severe responses might occur if experimental animals were exposed to 2-nitroaniline for a lifetime.

The screening chronic RfD of 0.015 mg/kg-day is higher than the chronic p-RfD of 0.004 mg/kg-day for 4-nitroaniline. As summarized before, the lack of data precludes a direct estimation of chronic RfD based on other nitroaniline isomers, and the lack of a POD based on critical hematological response significantly decreases the confidence in the critical study.

Confidence in the critical study is low because methemoglobin is not examined in the critical study. Confidence in the database is low due to lack of a development study in second animal species and the absence of a chronic study. However, the current database includes three developmental studies, and a reproductive study in rats. The overall confidence in the screening chronic RfD is low.

Screening Chronic RfC

The screening chronic RfC based on the $LOAEL_{HEC}$ of 0.459 mg/m^3 in male rats (Bio/Dynamics, 1983b) is derived as follows:

Screening Chronic RfC = $LOAEL_{HEC} \div UF$
= 0.459 mg/m^3 ÷ 10,000
= **0.00005 mg/m^3 or 5 × 10^{-5} mg/m^3**

The UF of 10,000 is composed of the same UFs used for subchronic p-RfC except for the use of a full, default UF of 10 for extrapolation from less-than-chronic to chronic exposure duration. A default 10-fold UF accounts for less-than-chronic exposure duration (duration of the critical study was only 4 weeks). There was neither subchronic nor chronic inhalation studies. This UF accounts for the possibility that incidence or severity of upper respiratory irritation could increase with longer exposure duration. The Bio/Dynamics (1983b) study supports the use of this UF by showing that the incidence of nasal irritation increased with exposure duration over the 4 weeks of the study (Table 4).

The screening chronic RfC of 0.00005 mg/m^3 is 120-fold lower than the chronic p-RfC of 0.006 mg/m^3 for 4-nitroaniline. As summarized above, use of the POD for 2-nitroaniline based on a more sensitive irritation response results in a lower screening chronic RfC value.

Confidence in the critical study is low to medium. Although the critical study had several shortcomings, such as only including two treated groups, encompassing a relatively short duration, including only males, limiting the histopathologic examination to tissues of the testes plus epididymides, another similar inhalation study provides some supportive evidence on the response observed. Confidence in the database is low due to lack of a comprehensive subchronic study, the lack of a comprehensive chronic study, and the lack of developmental and reproductive studies by inhalation route. Although no developmental or reproductive toxicity studies by the inhalation route are available, studies by the oral route suggest that 2-nitroaniline does not adversely affect reproduction or fetal development in the absence of maternal toxicity. The overall confidence in the screening chronic RfC is low.