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Provisional Peer Reviewed Toxicity Values for

4-Nitroaniline
(CASRN 100-01-6)

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

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 4-NITROANILINE (CASRN 100-01-6)

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

4-Nitroaniline or p-nitroaniline is an intermediate in the production of antioxidants, gasoline additives, and various dyes and pigments—including several azo dyes used for coloring consumer products (NTP, 1993). The empirical formula for 4-nitroaniline is $C_6H_6N_2O_2$ (see Figure 1).

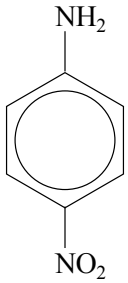

Figure 1. 4-Nitroaniline Structure

The U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2007) does not list a chronic oral reference dose (RfD), chronic inhalation reference concentration (RfC), or cancer assessment for 4-nitroaniline. Subchronic or chronic RfDs or RfCs for 4-nitroaniline are not listed in the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006); the HEAST cites inadequate data for quantitative risk assessment. The CARA list (U.S. EPA, 1991, 1994a) includes a Health and Environmental Effects Profile (HEEP) for nitroanilines (U.S. EPA, 1985), reporting insufficient data to support derivation of oral or inhalation toxicity value. The American Conference of Governmental Industrial Hygienists (ACGIH) (2001) established a TLV-TWA of 3 mg/m^3 for 4-nitroaniline, with a skin notation, to protect against anemia, anoxia, and liver effects resulting from methemoglobin formation. The National Institute for Occupational Safety and Health (NIOSH) (2005) established a REL-TWA of 3 mg/m^3 with a skin notation, to protect against effects in the respiratory system, blood, heart and liver. The Occupational Safety and Health Administration

(OSHA) (2007) lists an 8-hour TWA of 6 mg/m^3 for 4-nitroaniline, with a skin notation. ATSDR (2007). The International Agency for Research on Cancer (IARC) (2007) and the World Health Organization (WHO) (2007) have not published toxicological reviews on nitroanilines or 4-nitroaniline. Toxicity reviews on aromatic nitro, amino and nitro-amino compounds (Weisburger and Hudson, 2001; Woo and Lai, 2001) and the National Toxicology Program (NTP) (2007) management status and health and safety reports for 4-nitroaniline were consulted for relevant information.

Literature searches for studies relevant to the derivation of provisional toxicity values for 4-nitroaniline (CASRN 100-01-6) were conducted in MEDLINE, TOXLINE special, and DART/ETIC (1960s−December 2006); BIOSIS (2000−December 2006); TSCATS/TSCATS2, RTECS, CCRIS, HSDB, and GENETOX (not date limited); and Current Contents (June−December 2006). An update literature search (December 2006−October 2008) was conducted in MEDLINE.

REVIEW OF PERTINENT LITERATURE

Human Studies

No studies investigating the effects of subchronic or chronic oral exposure to 4-nitroaniline in humans were identified. Little information is available regarding inhalation exposure of humans to 4-nitroaniline. Anderson (1946) reported that ship workers who were exposed when sweeping spilled 4-nitroaniline powder (exposure occurred by both the inhalation and dermal routes) for approximately 8 hours became cyanotic, and complained of headache, somnolence, weakness, and respiratory distress. Hematological parameters were not assessed. Clinical signs greatly improved after intravenous injection of methylene blue, which is used for the treatment of methemoglobinemia. No quantitative data were located regarding the toxicity of 4-nitroaniline to humans following chronic or subchronic inhalation exposure.

Animal Studies

Oral Exposure

The effects of oral exposure of animals to 4-nitroaniline have been evaluated in subchronic (Monsanto Co., 1981a,b; Houser et al., 1983; NTP, 1993), chronic (NTP, 1993; Nair et al., 1990), developmental (Monsanto Co., 1979; 1980a,b; 1982), and reproductive (Nair et al., 1990) toxicity studies.

Short-term Study—Monsanto sponsored a short-term toxicity study of 4-nitroaniline oral toxicity in rats (Monsanto Co., 1981a). Groups of 10 male and 10 female Sprague-Dawley rats were fed diets adjusted to provide target 4-nitroaniline (purity 99.9%) doses of 0, 25, 50, 100, 250, or 500 mg/kg-day for 28 days. However, due to a calculation error, rats in the 250 and 500 mg/kg-day groups were substantially overdosed, with high-dose females actually receiving doses of 1440 mg/kg-day during weeks 2 and 3; the other treatment groups were overdosed, but to a lesser degree. Animals were examined daily for mortality and clinical signs, and body weights and food consumption were measured weekly. Necropsies were performed on all animals dying during the treatment period and on all animals surviving to the end of treatment. Mortality was observed in 7/10 females and 1/10 males in the 500 mg/kg-day group. Body

weight gain was significantly ($p \leq 0.01$) reduced by 9, 18, and 50% in males treated with 100, 250, and 500 mg/kg-day, respectively, and by 10, 13, 20, and 39% in females treated with 50, 100, 250, and 500 mg/kg-day, respectively; however, reduced food consumption and dosing errors confound interpretation of this finding. Clinical signs observed throughout the treatment period included yellow stained fur, squinting and tearing of the eyes, and paleness (indicative of anemia) in rats fed ≥100 mg/kg-day. During Week 4 of treatment, paleness was observed in all rats exposed to ≥100 mg/kg-day. Summary data of clinical observations over the 28-day treatment period were not reported. Necropsy examination revealed dose-related splenomegaly and spleen congestion in all treatment groups. Splenomegaly was observed in 0/10, 7/10, 9/10, 9/10, 10/10, and 9/9 males and 0/10, 2/10, 5/10, 10/10, 10/10, and 3/3 females in the 0, 25, 50, 100, 250, and 500 mg/kg-day groups, respectively; spleen congestion was observed in 0/10, 8/10, 9/10, 10/10, 10/10, and 9/9 males and 2/10, 10/10, 10/10, 10/10, 10/10, and 3/3 females in the 0, 25, 50, 100, 250 and 500 mg/kg-day groups, respectively. Clinical chemistry, hematology, and histopathologic evaluations were not performed. Because of the uncertainty associated with dosing, and because hematologic and histopathologic evaluations were not performed, this study is of limited use in risk assessment, and specific effect levels were not identified.

NTP (1993) sponsored a 14-day gavage study with 4-nitroaniline (purity >99%) in corn oil in B6C3F$_1$ mice. Groups of 5 male and 5 female mice were administered doses of 0, 10, 30, 100, 300, or 1000 mg/kg, 5 days/week for 2 consecutive weeks. The corresponding daily average doses were 0, 7.1, 21, 71, 214, and 714 mg/kg-day, respectively. Animals were observed for mortality and clinical signs twice daily. Body weights were recorded before treatment and on treatment days 7 and 14. Blood samples were obtained from all mice at the end of the treatment period and examined for hematology (hematocrit [Hct], Hgb, erythrocytes, reticulocytes, leukocyte counts with differential, total bone marrow cellularity and methemoglobin [an oxidized form of hemoglobin that does not bind oxygen]). All animals were necropsied and weights were recorded for 8 organs. Histopathology of comprehensive tissues (including gross lesions, tissue masses and associated lymph nodes, and 33 organs) was performed on all mice in the 300 mg/kg-day group. Although not reported in the methods section, the spleen was apparently examined histologically in some mice in the 100 mg/kg-day group as well.

All mice treated with 1000 mg/kg-day died within the first 4 days of treatment (NTP, 1993). The investigators attributed deaths to compound-related toxicity. Deaths of 6 other mice (3 males and 3 females) during the experiment were attributed to gavage error. The early deaths of all mice in the 1000 mg/kg-day group prevented assessment of any other endpoints in these mice. No treatment-related effects on body weight were observed in the 300 mg/kg-day and lower dose groups. Hematologic and pathologic findings in mice receiving 4-nitroaniline were characteristic of accelerated red blood cell (RBC) destruction caused by methemoglobin, with formation of Heinz bodies (inclusions within erythrocytes composed of denatured hemoglobin) and a compensatory response to maintain erythrocyte mass. Hematology results are shown in Table 1. Methemoglobin concentrations increased in a dose-dependent manner, with significant changes in all treatment groups compared to controls. Decreases in erythrocyte count and Hct occurred primarily at 30 mg/kg-day and above. Increased reticulocyte counts (indicating enhanced erythropoiesis) occurred in males treated with 300 mg/kg-day and in females treated with ≥30 mg/kg-day. Heinz bodies were observed in all male and female mice at 300 mg/kg-day and 2 males at 100 mg/kg-day. The researchers noted that increases in leukocyte count in the 100 and 300 mg/kg-day groups may have been, at least in part, an experimental artifact resulting from incomplete lysis of Heinz bodies and reticulocytes.

Table 1. Selected Hematology Parameters and Organ Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 14 Days[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)				
	0	**10 (7.1)**	**30 (21)**	**100 (71)**	**300 (214)**
Males					
Sample size	5	4	5	3	5
Organ Weights					
Absolute spleen (g)	0.121 ± .013[b]	0.118 ± 0.009 (98)	0.143 ± 0.012 (119)	0.191 ± 0.026 (158)[e]	0.359 ± 0.015 (297)[e]
Relative spleen (mg organ/g body wt)	4.46 ± 0.38	4.37 ± 0.27 (98)	5.06 ± 0.31 (113)	7.16 ± 0.81 (161)[e]	13.58 ± 0.41 (305)[e]
Absolute heart (g)	0.146 ± 0.003	0.152 ± 0.006 (104)	0.155 ± 0.007 (105)	0.152 ± 0.013 (104)	0.168 ± 0.004 (115)[d]
Relative heart (mg organ/g body wt)	5.40 ± 0.21	5.61 ± 0.21 (104)	5.50 ± 0.16 (102)	5.71 ± 0.43 (106)	6.35 ± 0.16 (118)[e]
Hematology					
Methemoglobin (%)	1.70 ± 0.22	3.03 ± 0.56 (178)[d]	5.74 ± 0.55 (337)[e]	13.77 ± 2.10 (810)[e]	11.92 ± 3.15 (701)[e]
Hematocrit (%)	43.0 ± 0.6	41.9 ± 0.7 (97)	39.0 ± 1.3 (91)[d]	42.7 ± 0.2 (99)	35.9 ± 1.7 (83)[e]
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.0 (97)	14.6 ± 0.5 (95)	19.0 ± 0.6 (123)	15.6 ± 0.8 (101)
RBC count (10^6/µL)	9.17 ± 0.15	9.00 ± 0.19 (98)	8.21 ± 0.29 (90)[d]	8.44 ± 0.06 (92)[d]	6.75 ± 0.32 (74)[e]
Reticulocyte count (10^6/µL)	2.90 ± 0.27	2.45 ± 0.70 (84)	3.32 ± 0.66 (114)	4.37 ± 1.78 (151)	18.04 ± 1.34 (622)[e]
Leukocyte count (10^3/µL)	4.22 ± 0.35	4.08 ± 0.34 (97)	4.22 ± 0.24 (100)	12.03 ± 4.63 (285)[d]	16.5 ± 3.38 (391)[e]
Heinz bodies	0[c]	0	0	2	5
Females					
Sample size	5	4	4	5	4
Organ Weights					
Absolute spleen (g)	0.109 ± 0.01[b]	0.118 ± 0.011 (108)	0.131 ± 0.013 (120)	0.184 ± 0.015 (169)[e]	0.300 ± 0.020 (185)[e]
Relative spleen (mg organ/g body wt)	4.91 ± 0.81	5.61 ± 0.47 (114)	5.74 ± 0.56 (117)	8.34 ± 0.57 (170)[e]	13.06 ± 0.90 (266)[e]

Table 1. Selected Hematology Parameters and Organ Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 14 Days[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)				
	0	**10 (7.1)**	**30 (21)**	**100 (71)**	**300 (214)**
Hematology					
Methemoglobin (%)	0 ± 0	1.35 ± 0.17[e]	3.2 ± 0.68[e]	6.17 ± 0.67[e]	16.73 ± 1.38[e]
Hematocrit (%)	43.4 ± 0.5	41.9 ± 0.9 (96)	42.6 ± 0.4 (98)	42.0 ± 1.2 (97)	36.2 ± 1.4 (83)[e]
Hemoglobin (g/dL)	15.4 ± 0.2	15.0 ± 0.4 (97)	15.5 ± 0.3 (101)	16.0 ± 0.3 (104)	17.5 ± 0.3 (114)[e]
RBC count (10^6/µL)	9.10 ± 0.09	8.78 ± 0.13 (96)[d]	8.80 ± 0.11 (97)	8.34 ± 0.24 (92)[e]	7.09 ± 0.25 (78)[e]
Reticulocyte count (10^6/µL)	0.80 ± 0.15	2.03 ± 0.67 (254)	2.73 ± 0.69 (341)[d]	4.92 ± 0.88 (615)[e]	5.95 ± 1.49 (744)[e]
Leukocyte count (10^3/µL)	2.90 ± 0.39	2.90 ± 0.35 (100)	3.00 ± 0.12 (103)	4.58 ± 0.13 (158)[e]	41.90 ± 4.21 (1445)[e]
Heinz bodies	0[c]	0	0	0	5

[a]NTP, 1993
[b]Means ± SE, () = percent of control
[c]Number of samples with Heinz bodies
[d]Significantly different from control ($p \leq 0.05$), Williams' or Dunnett's test (organ weights) or Dunn's or Shirley's test hematology performed by the researchers.
[e]$p \leq 0.01$

Selected organ weight results are shown in Table 1 (NTP, 1993). Absolute and relative spleen weights are increased in mice of both sexes treated with ≥100 mg/kg-day, and absolute and relative heart weights are increased in males treated with 300 mg/kg-day. At necropsy, all mice treated with 300 mg/kg-day and two males treated with 100 mg/kg-day had enlarged, purple spleens. Histological examination revealed the splenic red pulp of these mice to be filled with erythrocytes and precursors (indicating elevated hematopoiesis) and to contain many macrophages filled with hemosiderin. Hemosiderin was also found in Kupffer cells throughout the liver. Based on the development of methemoglobinemia in all treatment groups, a LOAEL of 10 mg/kg-day (daily average dose of 7.1 mg/kg-day) has been established for 2-week oral exposure to 4-nitroaniline; a NOAEL is not identified.

Subchronic Studies—Monsanto Co. (1981b) and Houser et al. (1983) reported a 90-day gavage study in which groups of 20 male and 20 female Sprague-Dawley rats were administered daily doses of 0, 3, 10, or 30 mg/kg-day of 4-nitroaniline (purity 99.85%) in corn oil. Animals were observed daily for mortality and clinical signs of toxicity. Body weights and food consumption were determined weekly. After 45 and again after 90 days of treatment, blood and urine samples were collected from 10 rats/sex/group for hematology, clinical chemistry, and urinalysis. At the end of the treatment period, all surviving animals were sacrificed and necropsied; selected organs were weighed, and histopathological examination was performed on comprehensive tissues.

No treatment-related mortalities occurred, with only one mortality in the control group (female) during the course of the study (Monsanto Co., 1981b; Houser et al., 1983). Body weight and food consumption were comparable to controls in all 4-nitroaniline treatment groups. Ear paleness (indicative of anemia) was observed in males treated with 30 mg/kg-day during treatment Week 2 (2/20 rats) and Week 4 (20/20) and in females treated with 30 mg/kg-day during treatment Weeks 2 (2/20 rats), Week 4 (20/20), and Week 6 (20/20). Ear paleness was not observed in any rats on other weeks during the treatment period. No other significant clinical signs of toxicity were observed. Clinical chemistry parameters in treatment groups were comparable to controls. Treatment-related effects on hematology parameters and histopathological findings were consistent with the effects of increased blood concentrations of methemoglobin; specifically, accelerated red blood cell (RBC) destruction (hemolytic anemia), and compensatory erythropoiesis to maintain erythrocyte mass. Methemoglobin concentration and reticulocyte count were significantly increased in all 4-nitroaniline treatment groups after 90 days of treatment (see Table 2). Other significant hematology findings in both sexes included decreased erythrocyte count, Hct, and blood hemoglobin concentration in males and females treated with ≥10 mg/kg-day, and decreased mean cell hemoglobin (MCH) and mean cell volume (MCV) in the 30 mg/kg-day group. Comprehensive histopathologic examination of the controls and 30 mg/kg-day rats identified the spleen as the only organ with treatment-related lesions; therefore, the spleens of all rats were examined microscopically. Dose-related increases in splenic congestion, hemosiderosis, and extramedullary hematopoiesis were observed in all treated groups (see Table 3). The LOAEL for 90-day oral exposure has been identified as a daily average dose of 3 mg/kg-day for the development of methemoglobinemia and associated hematological and splenic changes; a NOAEL is not established.

Table 2. Selected Hematology Parameters in Sprague-Dawley Rats Exposed to Oral 4-Nitroaniline for 45 or 90 Days[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	3	10	30
Males—45 days				
MetHgb (%)	1.0[b]	1.6 (160)[c]	3.2 (320)[c]	5.6 (560)[c]
Reticulocytes (%)	NR	NR	NR	NR
Males—90 days				
MetHgb (%)	1.14[b]	1.8 (164)[c]	3.3 (300)[c]	5.8 (527)[c]
Reticulocytes (%)	0.8	1.8 (225)[c]	3.1 (387)[c]	7.3 (912)[c]
Females—45 Days				
MetHgb (%)	1.0	1.7 (170)[c]	2.8 (280)[c]	4.4 (440)[c]
Reticulocytes (%)	NR	NR	NR	NR
Females—90 days				
MetHgb (%)	1.1	1.7 (154)[c]	2.8 (254)[c]	4.5 (409)[c]
Reticulocytes (%)	0.9	2.0 (222)[c]	3.4 (378)[c]	7.4 (822)[c]

[a]Monsanto Co., 1981b
[b]Mean, () = percent of control. NR = not reported in study.
[c]Significantly different from control ($p \leq 0.01$), Dunnett's test performed by the researchers

Table 3. Incidences of Selected Nonneoplastic Lesions of the Spleen in Rats Exposed to Oral 4-Nitroaniline for 90 Days[a]

Lesion Type	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	3	10	30
Males				
Excessive hemosiderin	0/20[b]	4/20	19/20[c]	20/20[c]
Hyperplasia of red pulp (reticulo-endothelial cells)	0/20	0/20	0/20	2/20
Excessive hematopoiesis	0/20	20/20[c]	20/20[c]	20/20[c]
Large cells with grey cytoplasm	0/20	2/20	13/20[c]	10/20[c]
Congestion	0/20	18/20[c]	17/20[c]	18/20[c]
Females				
Excessive hemosiderin	0/19	16/20[c]	20/20[c]	19/19[c]
Excessive hematopoiesis	0/19	19/20[c]	20/20[c]	19/19[c]
Large cells with grey cytoplasm	0/19	13/20[c]	7/20	15/19[c]
Congestion	0/19	5/20	17/20[c]	18/19[c]

[a]Monsanto Co., 1981b
[b]Number of rats with lesion/number of rats examined
[c]Significantly different from control ($p \leq 0.05$), Kolomogrov-Smirnov test performed by the researchers

In a 13-week study conducted by NTP (1993), groups of 20 $B6C3F_1$ mice/sex were administered 0, 1, 3, 10, 30, or 100 mg/kg-day 4-nitroaniline in corn oil, for 5 days/week, by gavage. The corresponding daily average doses were 0, 0.71, 2.1, 7.1, 21, and 71 mg/kg-day, respectively. After 7 weeks of treatment, 10−11 males and females in each group were sacrificed. The remaining animals were sacrificed at the end of the treatment period. Animals were evaluated as for the 14-day NTP study (NTP, 1993) with the following additions: (1) body weights were recorded initially, then weekly, and at termination; (2) necropsy performed at Week 7 included weight of the epididymis; (3) blood samples were analyzed from half the mice at day 45 and all surviving mice at termination (additional hematological parameters included MCV, MCH, mean cell hemoglobin concentration [MCHC]). Complete histopathology evaluation was performed on all mice sacrificed at 7 weeks and on all mice in the control and 100 mg/kg-day groups at termination. In addition, histopathology was performed on the liver in males and the spleen in both sexes from all dose groups.

Treatment with oral 4-nitroaniline had no adverse effect on survival or terminal body weights (NTP, 1993). Hematologic and pathologic findings at Week 7 and 13 were similar to those observed in the 14-day study and were primarily observed in the 30 and 100 mg/kg-day groups. Selected hematology parameters are shown in Table 4. Increased methemoglobin concentrations were noted in both sexes treated with ≥30 mg/kg-day for 7 weeks, in females treated with ≥30 mg/kg-day for 13 weeks, and in males treated with ≥10 mg/kg-day for 13 weeks. Increased treatment duration did not result in higher methemoglobin levels, with similar increases observed at 45 and 90 days within each dose group (see Table 4). Other hematologic evidence of erythrocyte destruction and regeneration was largely confined to the 30 and 100 mg/kg-day groups, except that reduced Hct was observed in females treated with ≥10 mg/kg-day for 7, but not 13, weeks.

NTP (1993) reported significantly increased absolute and relative spleen weights in a dose-related manner in both male and female mice in the 30 and 100 mg/kg-day groups at both the 7- and 13-week observation periods (see Table 5). Spleen weights in the 100 mg/kg-day group were more than double control values at both time points. Small increases (<20%) in absolute and/or relative liver weights were seen in mice of both sexes at 7 weeks, predominantly in the 30 and 100 mg/kg-day groups; no increases in liver weights were seen at 13 weeks. No effects on heart weight or weights of other organs were observed. Microscopic examination of tissues revealed compound-related increases in incidence and/or severity of splenic hemosiderosis and splenic/hepatic extramedullary erythropoiesis at the 7- and 13-week sacrifices in both sexes (see Table 6). The increases were most consistently observed in the ≥10 mg/kg-day dose groups, but significant increases were seen in all treated groups. Severity was minimal to slight in the lower dose groups, but moderate to marked in the 30 and 100 mg/kg-day groups. Hemosiderosis of hepatic Kupffer cells was observed in high-dose male mice at 7 and 13 weeks, but not in females at either time point. Although sporadic splenic histology findings were noted at lower doses, the weight of evidence of the histology, organ weight, and hematology data suggests that 10 mg/kg-day (daily average dose of 7.1 mg/kg-day) was a LOAEL in this study and 3 mg/kg-day (daily average dose of 2.1 mg/kg-day) a NOAEL.

Table 4. Hematology Parameters in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 7 or 13 Weeks[a]

Parameter	Exposure Group (daily average dose, mg/kg-day)					
	0	1 (0.71)	3 (2.1)	10 (7.1)	30 (21)	100 (71)
Males—7 Weeks						
n	9	8	8	9	9	8
MetHb (g/dL)	0.42 ± 0.11[b]	0.56 ± 0.10 (133)	0.53 ± 0.13 (126)	0.47 ± 0.09 (112)	1.25 ± 0.09[d] (296)	3.07 ± 0.31[d] (731)
Hct (%)	44.0 ± 0.7	45.6 ± 0.7 (104)	42.7 ± 1.0 (98)	44.0 ± 0.6 (100)	42.1 ± 0.9 (96)	41.3 ± 0.6[c] (94)
RBC (10^6/µL)	7.84 ± 0.12	8.15 ± 0.12 (104)	7.55 ± 0.14 (97)	7.89 ± 0.10 (101)	7.30 ± 0.14[c] (93)	7.08 ± 0.10[d] (90)
MCH (ρg)	17.7 ± 0.2	17.9 ± 0.1 (101)	18.0 ± 0.3 (102)	17.8 ± 0.2 (101)	19.7 ± 0.2[d] (113)	24.5 ± 0.3[d] (138)
MCHC (g/dL)	31.5 ± 0.3	32.0 ± 0.1 (102)	31.8 ± 0.2 (101)	32.0 ± 0.2 (102)	34.2 ± 0.3[d] (109)	42.0 ± 0.5[d] (133)
Reticulocytes (%)	2.64 ± 0.20	2.16 ± 0.25 (82)	1.88 ± 0.20 (71)	2.60 ± 0.31 (98)	4.58 ± 0.76 (173)	5.44 ± 0.41[d] (205)
Males—13 Weeks						
n	9	11	8	9	10	9
MetHb (g/dL)	0.36 ± 0.02	0.26 ± 0.02[d] (72)	0.29 ± 0.02 (81)	0.72 ± 0.03[c] (200)	0.74 ± 0.04[d] (206)	1.70 ± 0.20[d] (472)
Hct (%)	40.5 ± 0.7	45.8 ± 0.5 (113)	46.8 ± 1.1 (114)	41.2 ± 0.7 (102)	41.9 ± 0.5 (101)	39.7 ± 0.4 (98)
RBC (10^6/µL)	8.10 ± 0.14	8.89 ± 0.10 (110)	9.08 ± 0.18 (112)	8.03 ± 0.14 (99)	7.79 ± 0.10 (92)	7.56 ± 0.08[c] (93)
MCH (ρg)	16.5 ± 0.4	16.9 ± 0.2 (102)	17.2 ± 0.1 (104)	16.6 ± 0.2 (101)	19.3 ± 0.2[d] (117)	24.3 ± 0.3[d] (147)
MCHC (g/dL)	33.0 ± 0.4	32.9 ± 0.3 (100)	33.4 ± 0.2 (101)	32.4 ± 0.3 (98)	35.8 ± 0.4[d] (108)	46.2 ± 0.6[d] (140)
Reticulocytes (%)	2.56 ± 0.20	1.25 ± 0.19 (49)	1.80 ± 0.16 (70)	2.46 ± 0.28 (96)	5.86 ± 0.62[c] (230)	9.67 ± 0.86[d] (378)
Females—7 Weeks						
n	10	10	9	10	10	10
MetHb (g/dL)	0.06 ± 0.03	0.03 ± 0.03 (50)	0.04 ± 0.04 (67)	0.11 ± 0.03 (183)	0.42 ± 0.04[d] (700)	1.06 ± 0.11[d] (1767)
Hct (%)	49.0 ± 0.6	48.2 ± 0.3 (98)	47.6 ± 0.7 (97)	47.5 ± 0.4[c] (97)	42.4 ± 0.8[d] (86)	44.2 ± 0.7[d] (90)
RBC (10^6/µL)	8.39 ± 0.11	8.25 ± 0.09 (98)	8.25 ± 0.09 (98)	8.23 ± 0.07 (98)	7.42 ± 0.13[d] (88)	7.62 ± 0.11[d] (91)
MCH (ρg)	17.9 ± 0.1	17.8 ± 0.1 (99)	17.7 ± 0.1 (99)	17.7 ± 0.2 (99)	18.5 ± 0.1[c] (103)	20.2 ± 0.2[d] (113)

Table 4. Hematology Parameters in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 7 or 13 Weeks[a]

Parameter	Exposure Group (daily average dose, mg/kg-day)					
	0	**1 (0.71)**	**3 (2.1)**	**10 (7.1)**	**30 (21)**	**100 (71)**
MCHC (g/dL)	30.7 ± 0.1	30.5 ± 0.1 (99)	30.7 ± 0.1 (100)	30.6 ± 0.1 (99)	32.3 ± 0.2[d] (105)	34.9 ± 0.3[d] (114)
Reticulocytes (%)	2.02 ± 0.22	2.28 ± 0.32 (113)	1.81 ± 0.18 (90)	2.26 ± 0.22 (112)	4.64 ± 0.52[d] (230)	5.93 ± 0.39[d] (294)
Females—13 Weeks						
n	10	10	10	8	10	10
MetHb (g/dL)	0.37 ± 0.01	0.37 ± 0.04 (100)	0.23 ± 0.01 (62)	0.34 ± 0.02 (92)	1.01 ± 0.03 [d] (273)	1.47 ± 0.03[d] (397)
Hct (%)	40.8 ± 1.0	42.5 ± 0.4 (104)	43.7 ± 0.5 (107)	43.7 ± 0.5 (107)	44.2 ± 0.7 [c] (108)	39.9 ± 0.9 (98)
RBC (10^6/µL)	7.76 ± 0.18	8.14 ± 0.07 (105)	8.33 ± 0.09[c] (107)	8.33 ± 0.11 (107)	8.41 ± 0.14[c] (108)	7.70 ± 0.15 (99)
MCH (ρg)	17.0 ± 0.2	16.9 ± 0.1 (99)	17.2 ± 0.1 (101)	17.1 ± 0.1 (101)	17.0 ± 0.1 (1−00)	20.3 ± 0.3[d] (119)
MCHC (g/dL)	32.4 ± 0.3	32.3 ± 0.1 (100)	32.9 ± 0.1[c] (102)	32.5 ± 0.1 (100)	32.3 ± 0.2 (100)	39.3 ± 0.6[d] (121)
Reticulocytes (%)	1.64 ± 0.17	1.31 ± 0.19 (80)	1.39 ± 0.22 (85)	2.11 ± 0.36 (129)	4.44 ± 0.49[d] (271)	6.33 ± 0.41[d] (386)

[a]NTP, 1993
[b]Means ± SE, () = percent of control
[c]Significantly different from control ($p \leq 0.05$), Dunn's or Shirley's test performed by the researchers
[d]$p \leq 0.01$

Table 5. Absolute and Relative Spleen and Liver Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 7 or 13 Weeks[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
	0	**1 (0.71)**	**3 (2.1)**	**10 (7.1)**	**30 (21)**	**100 (71)**
Males—7 Weeks						
Sample size	9	8	8	9	9	8
Absolute spleen (g)	0.087 ± 0.004[b]	0.084 ± 0.003 (97)	0.087 ± 0.004 (100)	0.106 ± 0.009 (122)	0.142 ± 0.008[d] (163)	0.200 ± 0.010[d] (230)
Relative spleen (mg organ/g body wt)	3.02 ± 0.14	2.82 ± 0.11 (93)	2.91 ± 0.17 (96)	3.64 ± 0.37 (121)	4.88 ± 0.28[d] (162)	7.04 ± 0.03[d] (232)
Absolute liver (g)	1.404 ± 0.043	1.374 ± 0.044 (98)	1.564 ± 0.078 (112)	1.460 ± 0.028 (104)	1.576 ± 0.046 (112)	1.488 ± 0.049 (106)
Relative liver (mg organ/g body wt)	48.92 ± 1.10	45.98 ± 1.00 (94)	52.63 ± 2.69 (108)	49.58 ± 0.95 (101)	53.96 ± 0.84[c] (110)	52.39 ± 1.28[c] (107)
Males—13 Weeks						
Sample size	9	11	8	9	10	9
Absolute spleen (g)	0.091 ± 0.002	0.075 ± 0.003 (82)	0.084 ± 0.004 (92)	0.105 ± 0.004 (115)	0.147 ± 0.007[d] (162)	0.239 ± 0.008[d] (263)
Relative spleen (mg organ/g body wt)	2.82 ± 0.07	2.21 ± 0.09 (78)	2.64 ± 0.13 (94)	3.00 ± 0.11 (107)	4.53 ± 0.25[d] (161)	7.27 ± 0.26[d] (258)
Absolute liver (g)	1.614 ± 0.058	1.469 ± 0.033 (91)	1.508 ± 0.041 (93)	1.712 ± 0.046 (106)	1.649 ± 0.033 (102)	1.483 ± 0.047 (92)
Relative liver (mg organ/g body wt)	49.01 ± 1.20	43.15 ± 0.53[d] (88)	47.26 ± 0.79 (96)	48.93 ± 0.72 (100)	50.92 ± 1.04 (104)	44.91 ± 0.73[d] (92)
Females—7 Weeks						
Sample size	10	10	9	10	10	10
Absolute spleen (g)	0.105 ± 0.005	0.106 ± 0.002 (101)	0.113 ± 0.004 (108)	0.117 ± 0.003 (111)	0.177 ± 0.012[d] (169)	0.233 ± 0.011[d] (222)
Relative spleen (mg organ/g body wt)	4.24 ± 0.19	4.23 ± 0.07 (101)	4.56 ± 0.18 (108)	4.78 ± 0.16 (112)	7.00 ± 0.47[d] (165)	9.08 ± 0.45[d] (212)
Absolute liver (g)	1.179 ± 0.029	1.227 ± 0.018 (104)	1.248 ± 0.033 (106)	1.265 ± 0.036 (109)	1.306 ± 0.035[d] (111)	1.384 ± 0.038[d] (118)
Relative liver (mg organ/g body wt)	47.64 ± 1.04	49.18 ± 0.82 (103)	50.19 ± 1.09 (106)	51.67 ± 1.13[d] (108)	51.65 ± 1.20[d] (109)	53.89 ± 0.96[d] (113)
Females—13 Weeks						
Sample size	10	10	10	8	10	10
Absolute spleen (g)	0.097 ± 0.007	0.093 ± 0.004 (96)	0.101 ± 0.004 (104)	0.114 ± 0.010 (118)	0.141 ± 0.006[d] (145)	0.220 ± 0.009[d] (227)

Table 5. Absolute and Relative Spleen and Liver Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 7 or 13 Weeks[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
	0	**1 (0.71)**	**3 (2.1)**	**10 (7.1)**	**30 (21)**	**100 (71)**
Relative spleen (mg organ/g body wt)	3.65 ± 0.25	3.46 ± 0.14 (95)	3.69 ± 0.12 (101)	4.07 ± 0.27 (112)	5.00 ± 0.17[d] (137)	7.92 ± 0.39[d] (217)
Absolute liver (g)	1.354 ± 0.037	1.307 ± 0.030 (97)	1.364 ± 0.039 (101)	1.411 ± 0.062 (104)	1.432 ± 0.054 (106)	1.428 ± 0.026 (105)
Relative liver (mg organ/g body wt)	51.07 ± 1.03	48.74 ± 1.10 (95)	49.72 ± 1.33 (93)	50.74 ± 0.82 (99)	50.64 ± 1.38 (99)	51.16 ± 0.96 (100)

[a]NTP, 1993
[b]Means ± SE, () = percent of control
[c]Significantly different from control ($p \leq 0.05$), Williams' or Dunnett's test performed by the researchers
[d]$p \leq 0.01$

Table 6. Incidence of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 7 or 13 Weeks[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)					
	0	**1 (0.71)**	**3 (2.1)**	**10 (7.1)**	**30 (21)**	**100 (71)**
Males—7 Weeks						
Sample size	9	8	7	9	9	8
Liver: Kupffer cell pigmentation	0[b]	0	0	1 (0.4)	0	8[d] (3.2)[d]
Spleen: Extramedullary hematopoiesis	4 (0.9)	8[c] (1.8)[c]	7[e] (1.8)	9[c] (2.4)[d]	9[c] (2.1)[d]	8[c] (3.2)[d]
Spleen: Pigmentation	0	3 (0.4)	4[c] (0.5)[c]	9[d] (1.3)[d]	9[d] (2.0)[d]	8[d] (3.2)[d]
Males—13 Weeks						
Sample size	9	11	8	9	10	9
Liver: Kupffer cell pigmentation	0	0	0	0	1 (0.2)	9[d] (2.7)[d]
Liver: Extramedullary hematopoiesis	1 (0.4)	1 (0.2)	0	7[d] (2.2)[d]	10[d] (3.2)[d]	9[d] (3.9)[d]
Spleen: Pigmentation	0	0	0	3 (0.8)	10[d] (2.6)[d]	8[d] (1.8)[d]
Females—7 Weeks						
Sample size	10	10	9	10	10	10
Spleen: Extramedullary hematopoiesis	10 (2.5)	10 (2.5)	9 (2.8)	10 (2.7)	10 (3.6)[d]	10 (3.8)[d]
Spleen: Pigmentation	9 (1.7)	10 (1.9)	9 (1.9)	10 (2.2)[c]	10 (3.0)[d]	10 (3.0)[d]
Females—13 Weeks						
Sample size	10	10	10	8	10	10
Spleen: Extramedullary hematopoiesis	0	4[c] (0.9)[c]	1 (0.2)	5[d] (1.8)[d]	10[d] (2.9)[d]	9[d] (3.6)[d]
Spleen: Pigmentation	8 (1.6)	6 (1.2)	6 (1.2)	8 (2.1)	10 (2.9)[d]	9 (3.5)[d]

[a]NTP, 1993
[b]Number of responders, () = average severity grades for affected animals; 1 = minimal, 2 = slight, 3 = moderate, 4 = marked
[c]Significantly different from control ($p \leq 0.05$) by Fisher Exact test (incidence) or Mann-Whitney U test (severity score) performed by the researchers
[d]$p \leq 0.01$ in Fisher Exact test (incidence) or Mann-Whitney U test (severity score) performed by the researchers
[e]Not listed as statistically significant by NTP (1993), but found to be statistically significant ($p = 0.03$, two-tailed) by independent Fisher Exact test performed for this review

Chronic Studies—A 2-year chronic toxicity and carcinogenicity study was conducted by NTP (1993). Groups of 70 male and 70 female B6C3F$_1$ mice were gavaged with 0, 3, 30, or 100 mg/kg-day of 4-nitroaniline (purity >99%) in corn oil for 5 days/week. The corresponding daily average doses were 0, 2.1, 21, and 71 mg/kg-day, respectively. Mice were evaluated as described for the 13-week study (NTP, 1993) with the following changes. Body weights and clinical findings were recorded weekly for the first 13 weeks, monthly thereafter, and at termination. Groups of 10 mice/sex were scheduled for sacrifice after 9 and 15 months of treatment, when blood was analyzed for hematology and clinical chemistry (including methemoglobin and sulfhemoglobin [SulfHb]), and organ weight measurements were obtained for brain, right kidney, liver, spleen, and uterus (15 month measurement only). Complete histopathological examinations (including gross lesions, tissue masses, and associated lymph nodes, and 33 organs) were performed on all early deaths, all control and high-dose animals scheduled for interim evaluations, and all animals surviving to 2 years. Additional tissues evaluated at 9 months include liver, lung, spleen, and thyroid in all dose groups, the uterus in mid-dose females, and the urinary bladder and kidney of mid-dose males. Additional tissues evaluated at 15 months included liver and spleen in all dose groups, lung of mid-dose females, and bone marrow, lung, and stomach of mid-dose males. Hematology and clinical chemistry were not evaluated in mice terminated at 2 years.

Treatment with oral 4-nitroaniline had no effect on clinical observations, survival, or body weights throughout the study (NTP, 1993). Hematology and gross and microscopic findings are consistent with methemoglobinemia and compensatory erythropoiesis, as also observed in the 2- and 13-week studies (NTP, 1993). Table 7 presents selected hematology parameters measured after 9 and 15 months of exposure. Increased methemoglobin concentration was observed in mice of both sexes treated with ≥30 mg/kg-day. Increases in methemoglobin at 15 months were similar to, or lower than, increases observed at 9 months, indicating that increased exposure duration did not enhance the methemoglobin response, nor did it induce a significant response at lower doses (see Table 7). Levels of sulfhemoglobin (a partially oxidized and denatured mix of pigments resulting from nonspecific oxidative damage to red blood cells) were elevated in male mice treated with ≥30 mg/kg-day at the 9-month interval and in female mice treated with ≥30 mg/kg-day at the 9-month interval and 100 mg/kg-day at the 15-month interval. Reduced Hct and erythrocyte count were observed in mice of both sexes treated with 100 mg/kg-day and in female mice exposed to 30 mg/kg-day for 15 months. Evidence of enhanced erythropoiesis (increased reticulocyte count, mean cell hemoglobin, and mean cell hemoglobin concentration) was observed in mice of both sexes treated with ≥30 mg/kg-day at the 9-month evaluation.

Table 7. Selected Hematology Parameters in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9 and 15 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	3 (2.1)	30 (21)	100 (71)
Males—9 months				
Sample size	9	9	10	10
MetHb (g/dL)	0.2 ± 0.05[b]	0.23 ± 0.02 (115)	0.58 ± 0.06 (290)[d]	1.49 ± 0.16 (950)[d]
SulfHb (g/dL)	0.39 ± 0.05	0.46 ± 0.05 (118)	1.21 ± 0.17 (605)[d]	4.01 ± 0.56 (1028)[d]
Hct (%)	34.7 ± 1.0	34.0 ± 0.9 (98)	32.7 ± 0.6 (94)	31.8 ± 0.7 (92)[c]
Hgb (g/dL)	14.9 ± 0.3	14.8 ± 0.3 (99)	15.2 ± 0.2 (102)	16.4 ± 0.3 (110)[d]
RBC (10^6/µL)	9.16 ± 0.12	8.97 ± 0.12 (98)	8.89 ± 0.11 (98)	8.16 ± 0.09 (89)[d]
MCH (ρg)	16.2 ± 0.1	16.2 ± 0.2 (100)	17.1 ± 0.2 (106)[d]	20.1 ± 0.2 (124)[d]
MCHC (g/dL)	43.0 ± 0.5	43.4 ± 0.4 (101)	46.5 ± 0.3 (108)[d]	51.7 ± 1.3 (120)[d]
Reticulocytes (%)	0.12 ± 0.01	0.11 ± 0.02 (92)	0.23 ± 0.03 (192)[d]	0.38 ± 0.04 (317)[d]
WBC (103/µL)	0.67 ± 0.09	0.53 ± 0.06 (79)	1.13 ± 0.16 (169)	1.54 ± 0.21 (128)[d]
Males—15 months				
Sample size	10	10	10	10
MetHb (g/dL)	0.18 ± 0.03[e]	0.18 ± 0.04 (100)	0.34 ± 0.05 (189)[c]	0.82 ± 0.14 (456)[d]
SulfHb (g/dL)	0.43 ± 0.12[e]	0.35 ± 0.13 (81)	0.46 ± 0.16 (107)	1.26 ± 0.49 (293)
Hct (%)	33.3 ± 0.9	34.5 ± 1.2 (104)	30.0 ± 1.6 (91)	30.7 ± 0.8 (92)[c]
Hgb (g/dL)	13.2 ± 0.4	13.6 ± 0.4 (103)	12.7 ± 0.7 (96)	14.6 ± 0.3 (111)[d]
RBC (10^6/µL)	8.80 ± 0.29	8.86 ± 0.30 (101)	8.11 ± 0.50 (92)	7.79 ± 0.12 (86)[d]
MCH (ρg)	15.0 ± 0.2	15.4 ± 0.2 (103)	15.7 ± 0.3 (105)[d]	18.8 ± 0.4 (125)[d]
MCHC (g/dL)	39.5 ± 0.5	39.7 ± 0.5 (101)	42.3 ± 0.4 (107)[d]	47.7 ± 0.8 (121)[d]
Reticulocytes (%)	0.35 ± 0.06	0.30 ± 0.03 (86)	0.42 ± 0.05 (120)	0.85 ± 0.06 (143)[d]
WBC (103/µL)	1.78 ± 0.37	1.66 ± 0.27 (93)	1.40 ± 0.28 (79)	12.75 ± 2.05 (716)[d]
Females—9 months				
Sample size	9	10	9	10
MetHb (g/dL)	0.18 ± 0.06	0.20 ± 0.03 (111)	0.49 ± 0.12 (272)[d]	0.83 ± 0.12 (461)[d]
SulfHb (g/dL)	0.44 ± 0.05	0.46 ± 0.07 (105)	0.81 ± 0.09 (184)[d]	1.78 ± 0.25 (405)[d]
Hct (%)	33.7 ± 0.6	33.7 ± 0.7 (100)	34.0 ± 0.8 (101)	32.6 ± 0.7 (97)
Hb (g/dL)	14.6 ± 0.2	14.6 ± 0.2 (100)	15.0 ± 0.2 (103)	15.1 ± 0.3 (103)
RBC (10^6/µL)	8.97 ± 0.10	8.94 ± 0.09 (100)	8.96 ± 0.10 (100)	8.44 ± 0.13 (94)[c]
MCH (ρg)	16.3 ± 0.2	16.3 ± 0.1 (100)	16.8 ± 0.1 (103)[c]	17.9 ± 0.1 (110)[d]
MCHC (g/dL)	43.5 ± 0.6	43.4 ± 0.7 (100)	44.4 ± 0.7 (102)	46.5 ± 0.9 (107)[c]
Reticulocytes (%)	0.12 ± 0.02	0.13 ± 0.01 (108)	0.21 ± 0.02 (175)[d]	0.40 ± 0.04 (333)[d]
WBC (103/µL)	0.70 ± 0.10	0.59 ± 0.11 (84)	0.74 ± 0.18 (106)	0.75 ± 0.12 (107)

Table 7. Selected Hematology Parameters in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9 and 15 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	3 (2.1)	30 (21)	100 (71)
Females—15 months				
Sample size	8	10	10	9
MetHb (g/dL)	0.11 ± 0.03	0.31 ± 0.10 (282)	0.24 ± 0.04 (218)[c]	0.55 ± 0.07 (500)[d]
SulfHb (g/dL)	0.09 ± 0.05	0.24 ± 0.07 (267)	0.52 ± 0.17 (578)	0.86 ± 0.34 (956)[c]
Hct (%)	35.0 ± 0.7	33.7 ± 0.5 (96)	32.6 ± 0.7 (93)[c]	30.8 ± 0.5 (88)[d]
Hb (g/dL)	14.1 ± 0.3	13.5 ± 0.2 (96)	13.2 ± 0.2 (94)[c]	13.7 ± 0.2 (97)
RBC (10^6/µL)	9.09 ± 0.12	8.72 ± 0.12 (96)	8.44 ± 0.12 (93)[d]	7.81 ± 0.07 (86)[d]
MCH (ρg)	15.5 ± 0.1	15.4 ± 0.1 (99)	15.7 ± 0.1 (101)	17.5 ± 0.1 (113)[d]
MCHC (g/dL)	40.3 ± 0.5	40.0 ± 0.5 (99)	40.6 ± 0.5 (101)	44.4 ± 0.6 (110)[d]
Reticulocytes (%)	0.26 ± 0.02	0.30 ± 0.02 (115)	0.34 ± 0.03 (131)	0.78 ± 0.05 (300)[d,f]
WBC (103/µL)	0.66 ± 0.12	1.76 ± 0.58 (267)[d]	1.01 ± 0.17 (153)[c]	1.47 ± 0.31 (223)[d]

[a]NTP, 1993
[b]Means±SE, () = percent of control
[c]Significantly different from control ($p \leq 0.05$), Dunn's or Shirley's test performed by the researchers
[d]$p \leq 0.01$
[e]n = 9
[f]n = 8

Treatment-related effects on absolute and relative liver and spleen weights are provided in Table 8 (NTP, 1993). In male mice after 9 months of treatment, increased absolute liver weights were observed at a dose of 100 mg/kg-day and increased relative liver weights were observed at doses ≥30 mg/kg-day. Absolute and relative spleen weights were increased in male mice treated with 100 mg/kg-day. In female mice after 9 months of treatment, absolute, but not relative, liver weights were increased in the 100 mg/kg-day and absolute and relative spleen weights were increased in the 30 and 100 mg/kg-day groups. After 15 months of treatment, absolute and relative spleen weights in male mice were elevated in the 100 mg/kg-day group, but absolute and relative liver weights were comparable to controls. In females after 15 months of treatment, absolute and relative liver weights were elevated in 30 and 100 mg/kg-day groups and absolute spleen weight was elevated in the 100 mg/kg-day group. Treatment-related nonneoplastic lesions were observed in the bone marrow, liver, and spleen (see Table 9). Increased incidence of bone marrow hypercellularity, hemosiderosis of the Kupffer cells and spleen, splenic congestion, and splenic extramedullary erythropoiesis were observed. Histopathological findings were primarily observed at doses ≥30 mg/kg-day. Based on significant increases in the incidences of bone marrow hypercellularity in males and hemosiderosis of the spleen in females, a minimal chronic LOAEL of 3 mg/kg-day (daily average dose of 2.1 mg/kg-day) was identified; a NOAEL was not identified in this study. Although blood methemoglobin concentrations are not significantly increased in male or female mice in the 3 mg/kg-day group, the bone marrow hypercellularity and hemosiderosis of the spleen are consistent with methemoglobinemia-induced anemia and compensatory erythropoiesis.

Equivocal evidence for the carcinogenic activity of 4-nitroaniline was obtained in male mice, but not female mice (see Table 10). Significant positive trends were reported for the incidence of hemangiosarcomas in the liver and the incidence of vascular neoplasms (hemangiomas or hemangiosarcomas combined) at all sites in male mice. Although the incidences of these tumors were not significantly greater than the concurrent controls by pair-wise comparison, there were significant increases compared to historical controls. The incidence of hemangiosarcomas of the liver in the 100 mg/kg-day group was above the historical range and significantly different ($p < 0.05$, by Fisher exact test conducted for this review) from the historical control incidence. Similarly, the incidence in the 100 mg/kg-day group of hemangiomas and hemangiosarcomas at all sites was above the historical range and significantly different ($p < 0.01$, Fisher exact test) from the historical control incidence. The observations of hemangioma of the urinary bladder in 1 of the 10 male mice in the 100 mg/kg-day group at the 9-month sacrifice and of hemangiosarcoma of the liver in 1 of the 10 male mice in the 30 mg/kg-day group at the 15-month sacrifice (data not shown here), provide additional evidence that vascular tumors in the mice in this study may be compound related. The incidence of hemangioma or hemangiosarcoma (combined) at all sites was slightly elevated in female mice, but it was not significantly different from concurrent or historical controls. The NTP (1993) concluded that in female mice there was no evidence of carcinogenic activity of 4-nitroaniline, and that in male mice the evidence for carcinogenic activity was equivocal.

Table 8. Absolute and Relative Spleen and Liver Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9 and 15 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	**3 (2.1)**	**30 (21)**	**100 (71)**
Males—9 Months				
Sample size	10	10	10	10
Absolute liver (g)	1.795 ± 0.121[b]	1.956 ± 0.071 (109)	2.038 ± 0.076 (114)	2.202 ± 0.098 (123)[d]
Relative liver (mg organ/g body wt)	37.97 ± 1.74	39.02 ± 1.00 (103)	42.45 ± 0.94 (112)[c]	43.49 ± 1.37 (115)[d]
Absolute spleen (g)	0.085 ± 0.008	0.077 ± 0.003 (91)	0.103 ± 0.006 (121)	0.178 ± 0.009 (209)[d]
Relative spleen (mg organ/g body wt)	1.81 ± 0.15	1.54 ± 0.05 (85)	2.14 ± 0.11 (118)	3.52 ± 0.15 (194)[d]
Males—15 Months				
Sample size	9	10	10	10
Absolute liver (g)	1.998 ± 0.115	2.286 ± 0.097 (114)[e]	1.974 ± 0.122 (99)[f]	2.041 ± 0.132 (102)[e]
Relative liver (mg organ/g body wt)	39.37 ± 1.27	43.48 ± 1.63 (110)[e]	38.51 ± 1.69 (98)[f]	41.63 ± 1.21 (106)[e]
Absolute spleen (g)	0.078 ± 0.007	0.084 ± 0.006 (108)	0.136 ± 0.036 (174)	0.167 ± 0.009 (214)[d]
Relative spleen (mg organ/g body wt)	1.54 ± 0.13	1.61 ± 0.12 (105)	2.85 ± 0.86 (185)	3.44 ± 0.13 (223)[d]
Females—9 Months				
Sample size	9	10	9	10
Absolute liver (g)	1.466 ± 0.042	1.558 ± 0.051 (106)	1.580 ± 0.036 (108)	1.671 ± 0.037 (114)[d]
Relative liver (mg organ/g body wt)	33.88 ± 1.22	33.11 ± 0.84 (98)	35.58 ± 1.45 (105)	37.12 ± 1.26 (110)
Absolute spleen (g)	0.082 ± 0.005	0.092 ± 0.003 (112)	0.123 ± 0.009 (150)[d]	0.186 ± 0.004 (227)[d]
Relative spleen (mg organ/g body wt)	1.89 ± 0.09	1.96 ± 0.07 (104)	2.80 ± 0.24 (148)[d]	4.16 ± 0.21 (220)[d]

Table 8. Absolute and Relative Spleen and Liver Weights in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9 and 15 Months[a]

Parameter	Exposure Group (Daily Average Dose, mg/kg-day)			
	0	**3 (2.1)**	**30 (21)**	**100 (71)**
Females—15 Months				
Sample size	9	10	10	9
Absolute liver (g)	1.483 ± 0.058[f]	1.601 ± 0.065 (108)	1.676 ± 0.036 (113)[c]	1.774 ± 0.061 (120)[d]
Relative liver (mg organ/g body wt)	29.95 ± 1.24[f]	32.28 ± 0.68 (108)	33.30 ± 0.91 (111)[c]	33.80 ± 0.57 (113)[d]
Absolute spleen (g)	0.117 ± 0.025	0.103 ± 0.004 (88)	0.118 ± 0.006 (101)	0.199 ± 0.008 (170)[d]
Relative spleen (mg organ/g body wt)	2.66 ± 0.79	2.10 ± 0.12 (79)	2.34 ± 0.12 (88)	3.80 ± 0.15 (143)

[a]NTP, 1993
[b]Means ± SE, () = percent of control
[c]Significantly different from control ($p \leq 0.05$), Williams' or Dunnett's test performed by the researchers
[d]$p \leq 0.01$
[e]n = 9
[f]n = 8

Table 9. Incidences of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9, 15, or 24 Months[a]

Tissue and Lesion Type	Exposure Group (daily average dose, mg/kg-day)			
	0	3 (2.1)	30 (21)	100 (71)
Males—9 Months				
Bone marrow (hyperplasia)	0/10[b]	0/10	9/10[d]	10/10[d]
Liver (Kupffer cell pigmentation)	0/10	0/10	0/10	10/10[d]
Spleen (congestion)	0/10	0/10	6/10[d]	10/10[d]
Spleen (hematopoietic cell proliferation)	0/10	0/10	10/10[d]	10/10[d]
Spleen (hemosiderosis)	0/10	0/10	10/10[d]	10/10[d]
Males—15 Months				
Bone marrow (hyperplasia)	0/10	0/10	4/10[c]	9/10[d]
Liver (Kupffer cell pigmentation)	1/10	0/10	0/10	0/10
Spleen (congestion)	0/10	1/10	10/10[d]	10/10[d]
Spleen (hematopoietic cell proliferation)	2/10	0/10	10/10[d]	10/10[d]
Spleen (hemosiderosis)	0/10	0/10	10/10[d]	10/10[d]
Males—24 Months				
Bone marrow (hypercellularity)	1/50	10/50[d]	22/50[d]	27/50[d]
Liver (Kupffer cell pigmentation)	1/50	1/50	8/50[c]	50/50[d]
Spleen (hematopoietic cell proliferation)	13/50	18/50	37/50[d]	48/50[d]
Spleen (hemosiderosis)	0/50	1/50	46/50[d]	50/50[d]
Females—9 Months				
Liver (Kupffer cell pigmentation)	0/9	0/10	0/9	8/10[d]
Spleen (congestion)	0/9	0/10	9/9[d]	10/10[d]
Spleen (hematopoietic cell proliferation)	0/9	0/10	9/9[d]	10/10[d]
Spleen (hemosiderosis)	0/9	1/10	9/9[d]	10/10[d]
Females—15 Months				
Bone marrow (hyperplasia)	1/9	NA[e]	NA	0/9
Liver (Kupffer cell pigmentation)	1/9	0/10	0/10	0/9
Spleen (congestion)	0/9	2/10	7/10[d]	9/9[d]
Spleen (hematopoietic cell proliferation)	1/9	3/10	10/10[d]	9/9[d]
Spleen (hemosiderosis)	0/9	0/10	10/10[d]	9/9[d]

Table 9. Incidences of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice Exposed to Oral 4-Nitroaniline for 9, 15, or 24 Months[a]

Tissue and Lesion Type	Exposure Group (daily average dose, mg/kg-day)			
	0	3 (2.1)	30 (21)	100 (71)
Females—24 Months				
Bone marrow (hypercellularity)	6/52	4/50	8/51	22/51[d]
Liver (Kupffer cell pigmentation)	1/52	1/50	4/51	39/51[d]
Spleen (hematopoietic cell proliferation)	45/52	43/50	47/51	48/51
Spleen (hemosiderosis)	6/52	23/50[d]	45/51[d]	49/51[d]

[a]NTP, 1993
[b]Number of mice with lesion/number of mice examined
[c]Significantly different from control (0.05), Fisher Exact test (interims) or logistic regression test (termination) performed by the researchers
[d]$p \leq 0.01$
[e]Bone marrow not examined at these dose levels

Table 10. Incidence of Neoplastic Tumors in a 2-year Gavage Study of 4-Nitroaniline in Male and Female B6C3F$_1$ Mice[a]

Lesion Type		Exposure Group (Daily Average Dose, mg/kg-day)			
		0	3 (2.1)	30 (21)	100 (71)8
Males					
Hemangiosarcoma (liver)		0/50[b,c]	1/50 (2%)	2/50 (4%)	4/50[d] (8%)
Historical Incidence	Incidence	15/699			
	Mean (%)	2.1			
	Range (%)	0−6			
Hemangiosarcoma or Hemangioma (all sites)		5/50[c] (10%)	3/50 (6%)	4/50 (8%)	10/50[d] (20%)
Historical Incidence	Incidence	46/700			
	Mean (%)	6.6			
	Range (%)	0−12			
Females					
Hemangiosarcoma (liver)		1/52 (2%)	1/50 (2%)	0/51	0/51
Historical Incidence	Incidence	—			
	Mean (%)	—			
	Range (%)	—			
Hemangiosarcoma or Hemangioma (all sites)		1/52 (2%)	3/50 (6%)	3/51 (6%)	4/51 (8%)
Historical Incidence	Incidence	21/698			
	Mean (%)	3			
	Range (%)	0−12			

[a]NTP, 1993
[b]Number of mice with lesion/number of mice examined, —: not reported by NTP (1993)
[c]Statistically significant positive trend
[d]Statistically significant in pairwise test versus historical control ($p \leq 0.05$, Fisher exact test conducted for this review)

The effects of chronic oral exposure to 4-nitroaniline have been investigated in a 2-year gavage study in rats (Nair et al., 1990). Nair et al. (1990) treated groups of 60 male and 60 female Sprague-Dawley rats by daily gavage with 4-nitroaniline (purity 99.9%) in corn oil at doses of 0, 0.25, 1.5, or 9.0 mg/kg daily for 2 years. Rats were observed for mortality and clinical signs of toxicity twice daily, and were given detailed physical examinations weekly. Ophthalmoscopic examinations were conducted on all rats prior to treatment, and after 3, 12 and 24 months of treatment. Body weights and food consumption were recorded weekly for the first 14 weeks and biweekly thereafter. Hematology (MetHgb, Hgb, Hct, RBC count, reticulocyte count, WBC count with differential), serum chemistry (complete list not reported, but included serum sodium and potassium), and urinalysis (gross appearance, specific pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen and microscopic examination of sediment) were evaluated after 6, 10, 12, 18, and 24 months of treatment in randomly selected animals (10/sex/group); blood methemoglobin levels were evaluated at 6, 10, 12, 18, and 24 months. Complete necropsies were conducted on all animals. Organ weights of adrenals, brain, ovaries, testes, kidneys, liver, heart, and spleen were recorded for rats surviving at 2 years. Tissue masses, gross lesions, and tissue samples (35 tissues) were examined microscopically in all control and high-dose animals. In addition, all gross lesions and tissue masses, as well as the spleen and liver, were examined microscopically in low- and mid-dose animals.

Treatment resulted in slightly increased mortality in males treated with 9.0 mg/kg-day (44 deaths), relative to control (37 deaths) (Nair et al., 1990). Although the increase was not statistically significant by pairwise comparison, Life Table analysis showed a statistically significant positive trend for the males. Weekly mean body weights for 4-nitroaniline-treated males were similar to controls throughout the study. For females, weekly mean body weights were similar to controls for the 0.25 and 1.50 mg/kg-day groups, but tended to be higher than control values in the 9.0 mg/kg-day group, with differences reaching statistical significance at various times throughout the study (data not reported). Increased food intake occurred sporadically throughout the study in rats of both sexes treated with 1.5 or 9.0 mg/kg-day (data not reported). There were no treatment-related effects on clinical observations, ophthalmoscopic examinations, clinical chemistry, or urinalysis. Significant changes in hematological parameters attributed to 4-nitroaniline after 12 and 24 months of exposure are summarized in Table 11 (data from other time points not reported). Methemoglobin levels were increased in the 1.5 and 9.0 mg/kg-day groups at both time points in a dose-related manner in both sexes. In the high-dose groups, the increases in methemoglobin were large (6−8-fold over control levels) and methemoglobin levels exceeded 2%. Small decreases in hemoglobin and red blood cell count were also seen in the high-dose groups.

Table 11. Selected Hematology Parameters in Rats Exposed to Oral 4-Nitroaniline for 2 Years[a]

Parameter	Exposure Group (mg/kg-day)			
	0	0.25	1.50	9.00
Males—12 months				
Methemoglobin (%)	0.3 ± 0.2[b]	0.3 ± 0.2 (100)	0.8 ± 0.2 (267)[d]	2.4 ± 0.2 (800)[d]
Hemoglobin (g/100 mL)	15.5 ± 0.8	15.2 ± 0.6 (98)	15.5 ± 1.0 (100)	14.6 ± 0.5 (94)[c]
RBC count (10^6/μL)	8.19 ± 0.44	8.05 ± 0.38 (98)	8.06 ± 0.57 (98)	7.49 ± 0.29 (92)[d]
Males—24 months				
Methemoglobin (%)	0.3 ± 0.2	0.3 ± 0.2 (100)	0.7 ± 0.4 (233)[c]	2.5 ± 0.5 (833)[d]
Hemoglobin (g/100 mL)	14.6 ± 0.4	13.8 ± 1.5 (95)	13.8 ± 2.7 (95)	13.4 ± 2.4 (92)
RBC count (10^6/μL)	6.97 ± 0.25	6.59 ± 0.68 (95)	6.53 ± 1.04 (94)	6.10 ± 0.89 (88)[c]
Females—12 months				
Methemoglobin (%)	0.4 ± 0.2	0.4 ± 0.2 (100)	0.8 ± 0.3 (200)[d]	2.1 ± 0.2 (525)[d]
Hemoglobin (g/100 mL)	14.4 ± 0.6	14.4 ± 1.1 (100)	14.8 ± 0.6 (103)	13.9 ± 0.7 (97)
RBC count (10^6/μL)	6.95 ± 0.43	6.87 ± 0.45 (98)	7.05 ± 0.28 (101)	6.51 ± 0.28 (94)[c]
Females—24 months				
Methemoglobin (%)	0.4 ± 0.3	0.4 ± 0.3 (100)	0.8 ± 0.3 (200)[e]	2.6 ± 0.7 (650)[d]
Hemoglobin (g/100 mL)	13.9 ± 0.7	14.0 ± 0.9 (101)	14.2 ± 1.3 (102)	12.5 ± 1.6 (90)[c]
RBC count (10^6/μL)	6.07 ± 0.31	6.14 ± 0.46 (101)	6.21 ± 0.57 (102)	5.31 ± 0.86 (87)[c]

[a]Nair et al., 1990
[b]Means ± SD, () = percent of control, n = 10
[c]Significantly different from control ($p \leq 0.05$) as reported by the researchers
[d]$p \leq 0.01$
[e]Not listed as statistically significant in the report, but highly significant ($p = 0.008$) by independent analysis performed for this review (unpaired t-test)

In male rats, administration of 4-nitroaniline produced a dose-related increase in absolute and relative spleen weights in the 1.5 and 9.0 g/kg-day groups and increased relative liver weights in the 9.0 mg/kg-day group (see Table 12). Treatment did not affect absolute or relative organ weights in female rats. Microscopic examination revealed increased accumulations of brown pigment (probably hemosiderin) in the Kupffer cells (sinusoidal macrophages) of the liver and reticuloendothelial cells of the spleen of treated rats (see Table 12). Statistical analysis of data was not performed by the study authors. Fisher Exact tests performed for this review showed that the increases were statistically significant in the liver in the high-dose groups of both sexes and in the 1.5 mg/kg-day group in males. The incidence of hemosiderosis in the spleen was significantly increased in males of the 1.5 and 9.0 mg/kg-day groups. Due to the high incidence of splenic hemosiderosis in control females, there was no increase in overall incidence with treatment. However, the severity of splenic hemosiderosis increased with dose in both sexes. The Jonckheere-Terpstra test performed for this review showed that the increase in severity was statistically significant at ≥0.25 mg/kg-day in the female rats. The same pattern was seen in the male rats, although the increase in severity in males was not statistically significant at doses lower than 9.0 mg/kg-day. Based on increased methemoglobin in both male and female rats, and increases in spleen weights and hemosiderosis in the liver and spleen in male rats, the NOAEL and LOAEL in this study were 0.25 mg/kg-day and 1.5 mg/kg-day, respectively.

Several types of spontaneously occurring neoplasms were observed in rats of all groups; however, the incidence of all tumor types was similar in 4-nitroaniline-treated and control rats, with no dose-related increase in incidence. The most commonly observed tumors occurred in the pituitary of both sexes and in the mammary gland of the females. The investigators concluded that there was no evidence of a carcinogenic effect of oral 4-nitroaniline in rats at the dose levels used in this study. The study authors also noted the absence of splenic fibrosis, which has been identified as a nonneoplastic precursor to the development of rare splenic tumors in rats treated with aniline or aniline analogs (Goodman et al., 1984).

Table 12. Spleen and Liver Weights and Microscopic Changes in Rats Exposed to Oral 4-Nitroaniline for 2 years[a]

Parameter	Exposure Group (mg/kg-day)			
	0	0.25	1.50	9.00
Males				
Absolute spleen weight (g)	0.869 ± 0.127[b]	0.923 ± 0.186 (106)	0.948 ± 0.169 (109)	1.119 ± 0.213 (129)[f]
Relative spleen weight ([organ wt/body wt] × 100])	1.17 ± 0.19	1.32 ± 0.37 (113)	1.46 ± 0.38 (125)[e]	1.60 ± 0.36 (162)[f]
Absolute liver weight (g)	17.2 ± 3.7	17.2 ± 3.0 (100)	17.2 ± 3.6 (100)	19.5 ± 4.5 (113)
Relative liver weight ([organ wt/body wt] × 100)	2.28 ± 0.28	2.41 ± 0.34 (106)	2.61 ± 0.62 (114)	2.84 ± 1.10 (125)[e]
Liver brown pigment (incidence)	18/60[c]	18/60	28/60[g]	44/60[g]
Spleen brown pigment (incidence)	37/60	43/60	50/60[g]	47/60[g]
Spleen brown pigment (degree of pigmentation)				
Slight	23[d]	17	16	4[h]
Moderate	8	14	16	18[h]
Moderately severe	6	12	18	25[h]
Females				
Liver brown pigment (incidence)	38/60[c]	28/59	40/60	54/60[g]
Spleen brown pigment (incidence)	50/60	55/60	54/60	54/59
Spleen brown pigment (degree of pigmentation)				
Slight	15[d]	8[h]	2[h]	4[h]
Moderate	16	18[h]	7[h]	12[h]
Moderately severe	19	29[h]	45[h]	38[h]

[a]Nair et al., 1990
[b]Means ± SE, () = percent of control
[c]Number of rats with finding/number of rats examined
[d]Number of responders
[e]Significantly different from control ($p \leq 0.05$), as reported by the researchers
[f]Significantly different from control ($p \leq 0.01$), as reported by the researchers
[g]Significantly different from control ($p \leq 0.05$) by Fisher Exact test performed for this review
[h]Significantly different from control ($p \leq 0.05$) by Jonckheere-Terpstra test performed for this review

Developmental and Reproduction Studies—4-Nitroaniline was tested for developmental toxicity in rabbits (Monsanto Co., 1980a, 1982) and rats (Monsanto Co., 1979, 1980b), and for reproductive toxicity in rats (Nair et al., 1990). Monsanto Co. conducted a range-finding developmental study (1980a) and a definitive developmental study (1982) in rabbits. In the range-finding developmental toxicity study, Monsanto Co. (1980a) administered 4-nitroaniline (purity 99.89%) in corn oil at doses of 0, 25, 85, 250, or 625 mg/kg-day administered by gavage on gestational days (GD) 7−19 to groups of 5 New Zealand white rabbits. Does were examined daily for mortality and signs of toxicity, and body weights were recorded on GD 0, 7, 19, and 30. Detailed physical examinations were performed on GD 0, 7, 10, 15, 19, 25, and 30. Animals were sacrificed on GD 30 and evaluated for implantations, resorptions, and live or dead fetuses. Necropsies were performed on all surviving does on GD 30. Fetuses were examined for body weight and gross external malformations. At the 250 and 625 mg/kg-day dose levels, mortality among does was 80 and 100%, respectively; deaths occurred on GD 9−12. Although the cause of death was not reported, the authors observed hemorrhages of the trachea and esophagus, and fluid-filled lungs. No adverse effects on maternal survival, body weight, implantations, or resorptions were observed in rabbits treated with 25 or 85 mg/kg-day. Fetal weights and the incidence of external malformations in the 25 and 85 mg/kg-day groups were similar to controls. Fetuses from the one 250 mg/kg-day doe that survived to termination were not evaluated. Results identify 85 mg/kg-day as a NOAEL for maternal and fetal effects and 250 mg/kg-day as a FEL for rabbits exposed during gestation. However, the absence of histopathologic evaluation of does dictates caution in interpreting the maternal NOAEL, given the pronounced mortality at the next higher dose.

In its definitive rabbit developmental study, Monsanto Co. (1982) treated groups of 18 does with 4-nitroaniline (purity not reported) at doses of 0, 15, 75, or 125 mg/kg-day in corn oil on days 7−19 of gestation. Evaluations of does were conducted as in the range-finding developmental study (Monsanto Co., 1980a), but fetuses were also examined for skeletal and internal malformations. Treatment-related mortalities among the does (7/18) occurred in the 125 mg/kg-day, but not the 15 or 75 mg/kg-day groups. Other non-treatment related deaths were reported in the control group (1/18) and the 75 mg/kg-day group (1/18). Yellowish staining of the fur in the anogenital area was observed in all 4-nitroaniline treatment groups. No treatment related maternal effects were observed for body weight or reproductive outcome in the 15 and 75 mg/kg-day groups. In the 125 mg/kg-day group, increased incidence of does with body weight loss (control—50% of does lost weight; 125 mg/kg—97.1% of does lost weight) was observed, but mean body weight change over the treatment period was not different in treated does compared to controls. Increased, but not statistically significant, incidence of abortion was observed in the 125 mg/kg group (control—0/18 does; 125 mg/kg—3/18 does). Pregnancy, implantation, and resorption rates in all treatment groups were similar to the controls. No treatment-related effects were observed for fetal body weight, sex distribution, or the incidence of skeletal, external, or visceral anomalies. The NOAEL and LOAEL values for maternal toxicity (mortality and incidence of weight loss) were 75 and 125 mg/kg-day (daily average), respectively. A NOAEL of 125 mg/kg-day (daily average) was identified for developmental effects; a LOAEL was not identified.

Monsanto Co. also conducted range-finding (Monsanto Co., 1979) and definitive developmental studies (Monsanto Co., 1980b) in rats. In the range-finding developmental study, groups of 5 Sprague-Dawley rats were administered 4-nitroaniline (purity not reported) in corn oil at doses of 0, 5, 20, 80, 325, or 1300 mg/kg-day by gavage on GD 6−19 (Monsanto Co., 1979). Dams were examined daily for mortality and signs of toxicity, and body weights were

recorded on GD 0, 6, and 20. Detailed physical examinations were performed on GD 0, 6, 10, 15, and 20. Animals were sacrificed on GD 20 and evaluated for implantations, resorptions, and live or dead fetuses. Necropsies were performed on all surviving dams on GD 20. Fetuses were examined for body weight and gross external malformations. All rats in the 1300 mg/kg-day group died, or were sacrificed due to moribund conditions, within the first 3 days of treatment. No mortalities occurred in control or other 4-nitoaniline treatment groups. Clinical signs of toxicity in dams were limited to cyanosis at dose levels of 20, 80, and 325 mg/kg and moderate alopecia at 325 mg/kg. Maternal weight gain was similar to controls at doses ≤80 mg/kg-day, but was decreased by 29% (statistical significance not reported) in the 325 mg/kg-day group compared to controls. Splenomegaly was observed in 3/5 and 5/5 dams in the 80 and 325 mg/kg-day, respectively, compared to 0/5 rats in the control group; the incidence of splenomegaly was significantly ($p \leq 0.05$; Fisher's Exact Text conducted for this review) increased compared to controls in the 325 mg/kg-day group. The mean number of resorptions was increased in dams treated with 325 mg/kg-day (11.4) compared to controls (0.6) (statistical significance not reported). No external fetal malformations were observed in controls or animals treated with ≤80 mg/kg-day; however, in the 325 mg/kg-day group, a total of 15 fetuses from 3 dams had malformations of the tail and digits. The NOAEL and LOAEL values for maternal toxicity (cyanosis) were identified as 5 and 20 mg/kg-day, respectively. For developmental effects (tail and digit malformations), the NOAEL and LOAEL values are 80 and 325 mg/kg-day, respectively.

In its definitive rat developmental study, Monsanto Co. (1980b) treated groups of 24 Sprague-Dawley dams with 4-nitroaniline (purity 99.89%) at doses of 0, 25, 85, or 250 mg/kg in corn oil on days 6−19 of gestation. Evaluations of dams were conducted as in the range-finding developmental study (Monsanto Co., 1980b), but fetuses were also examined for skeletal and internal malformations and maternal spleen weights were recorded. No mortalities were observed in the control or 4-nitroaniline treated rats. Maternal weight gain was significantly reduced compared to controls in the 250 mg/kg-day group (45% decrease, $p \leq 0.01$), but not in other treatment groups. Maternal effects include pale eye color, convulsions, and increased resorptions in rats receiving 250 mg/kg-day. Yellow staining of the anogenital fur and increased incidence of gross discoloration and surface irregularities of the spleen were observed in dams receiving ≥85 mg/kg-day. In dams treated with ≥85 mg/kg-day, significant increases ($p \leq 0.01$) in absolute spleen weight (171 and 200% of controls in 85 and 250 mg/kg-day groups, respectively) and relative spleen weight (165 and 204% of controls in 85 and 250 mg/kg-day groups, respectively) were observed. The percentage of resorptions was significantly increased in the high-dose group (control—3.8%; 250 mg/kg—14.2%; $p \leq 0.01$) and the percentage of live fetuses was significantly reduced (control—96.2%; 250 mg/kg—85.8%; $p \leq 0.01$). No treatment-related effects on uterine implantations or number of live fetuses were observed in the 25 or 85 mg/kg-day groups. A dose-related decrease in male and female fetal weight, compared to control, was observed, with changes reaching statistical significance in the 85 (males, 6.4% decrease; females 6.4% decrease; $p \leq 0.05$) and 250 mg/kg-day (males, 27.1% decrease; females 29.6% decrease; $p \leq 0.01$) groups. The incidences of fetal external and internal malformations were comparable to controls at dose levels ≤85 mg/kg-day groups, and increased in the 250 mg/kg-day group. External malformations were primarily of the tail and digits, and were observed in 20% of high-dose fetuses (in 13/22 litters), compared to 0.3% of controls ($p \leq 0.01$). Internal malformation of the kidneys was observed in 9.8% of fetuses (in 5/22 litters) in the high-dose group; malformations of other soft tissues were not observed. No internal malformations were observed in controls. Skeletal variations (primarily ossification variations) were observed in 85 and 100% of fetuses in the control and high-dose groups

($p \leq 0.01$); variations were observed in all control and high-dose litters. The NOAEL and LOAEL values were identified as 25 and 85 mg/kg-day for both maternal (alterations of spleen weight and gross appearance of spleen) and fetal effects (decreased fetal weight).

Nair et al. (1990) performed a 2-generation reproductive toxicity study in Sprague-Dawley rats with 4-nitroaniline (purity 99.9%) in corn oil. Groups of 15 males and 30 females were treated daily for 14 (F_0) or 18 weeks (F_1) before mating and during mating, gestation, and lactation. The gavage doses of 0, 0.25, 1.5, and 9.0 mg/kg-day were the same as used in the chronic toxicity study (Nair et al., 1990) summarized earlier. Observations made during the study included mortality, clinical signs of toxicity, body weight, food consumption, mating and fertility indices, pup and litter survival indices, and the histopathology of selected tissues (not specified) from the F_0 and F_1 adults and F_1 and F_2 pups. Treatment with oral 4-nitroaniline had no effect on survival, clinical signs, food consumption, or body weight of the parental generations. Pregnancy rate was significantly reduced compared to controls (control: 95.7%; 9 mg/kg: 66.7%; $p \leq 0.05$) in the F_0 but not the F_1, generation. Because this effect was not observed in the F_1 generation, the authors did not consider decreased pregnancy rate in F_0 to be a treatment-related effect. There were no adverse effects on gestation length, litter size, offspring survival or body weight, or on the morphologic appearance of the testes of F_0 adults or selected tissues of F_1 adults or F_1 or F_2 offspring. Thus, a freestanding NOAEL of 9.0 mg/kg-day was identified for reproductive effects; a LOAEL was not determined.

Inhalation Exposure

Short-term Studies—Short-term inhalation toxicity studies of 4-nitroaniline, with exposure durations of 2 to 4 weeks, were conducted by Nair et al. (1986) and DuPont Co. (1994). The effects of inhalation exposure of rats to 4-nitroaniline for 4 weeks was studied by Nair et al., (1986). Groups of 10 male (204−243 g) and 10 female (204−243 g) Sprague-Dawley rats were exposed (whole body exposure) to an aerosol of 4-nitroaniline 6 hours/day, 5 days/week, for 4 weeks. 4-Nitroaniline was dissolved in isopropyl alcohol and the solution fed into a spray atomizer. Mean measured exposure concentrations for 4-nitroaniline were 0 (1500 ppm solvent only), 10, 32, and 80 mg/m^3. Particle size mass median aerodynamic diameters and geometric standard deviations (MMAD ± GSD) were 0.80 ± 5.42, 1.37 ± 4.04 and 0.78 ± 6.42 μm for the 10, 32, and 80 mg/m^3 exposures, respectively. Endpoints monitored throughout the study include mortality, clinical signs, and body weights. A comprehensive ophthalmoscopic examination was performed on all rats before the study began and prior to termination of the study. Blood was drawn from all animals before sacrifice for hematologic and clinical chemistry determinations. At the end of the study, all rats underwent gross necropsy and the major organs were weighed. Microscopic examinations of all major organs and tissues (including nasal turbinates, trachea, and lungs) of all control and high-exposure rats, and of spleens of all rats, were performed.

No mortality or compound-related clinical signs of toxicity were observed during the study, and body weights were not different from controls (data not reported) (Nair et al., 1986). Results from the ophthalmoscopic examinations showed no treatment-related changes. Hematologic changes attributed to exposure to 4-nitroaniline were: a concentration-related increase in blood methemoglobin (MetHb) levels in male and female rats that was statistically significant at ≥32 mg/m^3; an increased incidence of morphological changes in the red blood cells (polychromasia in both sexes and anisocytosis in females) at ≥32 mg/m^3 (incidence data and statistical significance not reported); and significantly increased WBC counts in males at

80 mg/m^3 (see Table 13). Data on RBC counts were not reported. These changes in hematological parameters are consistent with 4-nitroaniline-induced methemoglobinemia and compensatory hematopoiesis. No treatment-related clinical chemistry findings or gross pathological changes were observed. Increased relative and absolute spleen weights were observed in males and females in all 4-nitroaniline groups (see Table 14). Hemosiderosis and extramedullary hematopoiesis in the spleen were observed in all groups with comparable frequency; however, the severity of the changes was concentration-related (see Table 14). Livers of the high-exposure females had a qualitatively higher degree of extramedullary hematopoiesis relative to the controls (data not reported). No compound-related histopathological changes were observed in other tissues. A LOAEL of 10 mg/m^3 was identified for increased spleen weights and severity of splenic hemosiderosis and extramedullary hematopoiesis in males and females. The corresponding human equivalent concentration (HEC)[1] is 4.2 mg/m^3 for the systemic toxicity. A NOAEL was not identified.

Table 13. Effect of Inhalation Exposure of Male and Female Rats with 4-Nitroaniline (4-Week Exposure) on Hematological Parameters[a]

Parameter	Exposure Group (mg/m^3)			
	0	**10**	**32**	**80**
Males				
MetHb (%)	1.5 ± 0.8[b]	2.8 ± 1.4 (187)	3.6 ± 1.1 (240)[d]	5.5 ± 2.1 (367)[d]
WBC (10^3/µL)	11.7 ± 2.3	15.0 ± 6.0 (128)	14.9 ± 4.4 (127)	19.4 ± 6.5 (169)[c]
Females				
MetHb (%)	1.4 ± 1.0	1.4 ± 1.1 (100)	3.1 ± 1.4 (221)[d]	3.8 ± 1.3 (271)[d]
WBC (10^3/µL)	10.5 ± 3.0	9.1 ± 3.0 (87)	10.6 ± 4.1 (101)	9.0 ± 1.9 (86)

[a]Nair et al., 1986
[b]Means ± SD, () = percent of control, 10 males and 10 females per treatment group
[c]Significantly different from control ($p \leq 0.05$), as reported by the researchers
[d]Significantly different from control ($p \leq 0.01$), as reported by the researchers

[1] The detailed calculation of the human equivalent concentration can be found in the section of Derivation of Provisional Subchronic and Chronic Inhalation RfCs for 4-Nitroaniline.

Table 14. Effect of Inhalation Exposure of Male and Female Rats with 4-Nitroaniline (4-Week Exposure) on Spleen Weight and Histopathology Findings[a]

Parameter	Exposure Group (mg/m^3)			
	0	**10**	**32**	**80**
Males				
Absolute spleen weight (g)	0.57 ± 0.06[b]	0.71 ± 0.17 (125)[d]	0.70 ± 0.10 (123)	0.87 ± 0.16 (153)[e]
Relative spleen weight ([organ wt/body wt] × 100)	0.18 ± 0.02	0.23 ± 0.05 (128)	0.23 ± 0.04 (128)[d]	0.28 ± 0.05 (156)[e]
Hemosiderosis				
Minimal	8[c]	3[f]	0[f]	0[f]
Mild	1	5[f]	0[f]	0[f]
Moderate	1	2[f]	2[f]	1[f]
Marked	0	0[f]	6[f]	8[f]
Massive	0	0[f]	2[f]	1[f]
Extramedullary Hematopoiesis				
Minimal	1[c]	1[f]	0[f]	0[f]
Mild	9	1[f]	3[f]	1[f]
Moderate	0	7[f]	7[f]	3[f]
Marked	0	1[f]	0[f]	6[f]
Females				
Absolute spleen weight (g)	0.52 ± 0.07[b]	0.71 ± 0.20 (136)[d]	0.64 ± 0.1 (123)	0.72 ± 0.13 (138)[e]
Relative spleen weight ([organ wt/body wt] × 100)	0.25 ± 0.03	0.34 ± 0.09 (136)[e]	0.31 ± 0.04 (124)	0.34 ± 0.06 (136)[e]
Hemosiderosis				
Minimal	3[c]	0[f]	0[f]	0[f]
Mild	7	4[f]	0[f]	0[f]
Moderate	0	6[f]	0[f]	0[f]
Marked	0	0[f]	5[f]	4[f]
Massive	0	0[f]	5[f]	6[f]

Table 14. Effect of Inhalation Exposure of Male and Female Rats with 4-Nitroaniline (4-Week Exposure) on Spleen Weight and Histopathology Findings[a]

Parameter	Exposure Group (mg/m^3)			
	0	**10**	**32**	**80**
Extramedullary Hematopoiesis				
Minimal	1[c]	0	0[g]	0[g]
Mild	3	3	0[f]	0[f]
Moderate	4	3	4[f]	2[f]
Marked	0	4	6[f]	8[f]

[a]Nair et al., 1986
[b]Means ± SD, () = percent of control, 10 males and 10 females per treatment group
[c]Number of responders
[d]Significantly different from control ($p \leq 0.05$), as reported by the researchers
[e]Significantly different from control ($p \leq 0.01$), as reported by the researchers
[f]Significantly different from control ($p \leq 0.05$) by Jonckheere-Terpstra test performed for this review

The effects of short-term inhalation of 4-nitroaniline to rats was also reported in an unpublished study (DuPont Co., 1994). Groups of 16 male Crl:CD(SD)BR rats were exposed (head-only) to 0, 0.05, 0.51, or 1.12 mg/L (equivalent to 0, 50, 510, or 1120 mg/m^3) for 6 hours per day, 5 days per week, for 2 weeks. Particle size mass median aerodynamic diameter and geometric standard deviations were 2.6 ± 3.1 and 2.4 ± 2.4 µm for the 510 and 1120 mg/m^3 groups, respectively; particle size was not determined in the 50 mg/m^3 group. Mortality, clinical signs and body weight were monitored throughout the study. At the end of the exposure period, urine and blood samples were collected from 10 rats per group for analysis of hematology and clinical chemistry parameters and urinalysis. Of these 10 rats/group, gross and histopathological examinations on comprehensive tissues (including nose, trachea and lungs) were performed on 5 rats per/group at the end of the 2-week exposure period. Organ weights were measured for heart, kidneys, liver, lungs, spleen, testes, and thymus. The six rats not sacrificed after 2 weeks in each exposure group were allowed to recover for 14 days following the exposure period. Approximately every other day during recovery and at the end of the recovery period, blood samples were collected for analysis. At the end of the recovery period, gross and histopathological examinations on comprehensive tissues were performed.

No mortalities were observed during the treatment or recovery periods in any group (DuPont Co., 1994). With the exception of yellow-stained fur in rats of all 4-nitroaniline-exposed groups, no clinical signs of toxicity were observed during the exposure or recovery periods. At the end of the treatment period, mean body weight was significantly decreased in the 1120 mg/m^3 group, but not the 50 or 510 mg/m^3 groups, compared to controls (see Table 15). Results of blood analysis, gross pathology, and histopathology were consistent with the development of hemolytic anemia, methemoglobinemia, and compensatory hematopoietic and leukopoietic responses. Selected hematology and clinical chemistry parameters are shown in Table 16. At the end of the treatment period, a significant dose-dependent increase in methemoglobin levels was observed in all treatment groups (only group means reported). Treatment-related effects were observed on several hematology parameters, including MCV, MCH, and MCHC in the 1120 mg/m^3 group, and Hgb, RBC, and WBC counts in the 510 and 1120 mg/m^3 groups. Serum cholesterol was elevated in all 4-nitroaniline groups, although the study authors did not discuss the clinical relevance of the observed increases. Rats exposed to 510 and 1120 mg/m^3 also excreted less concentrated urine with a higher pH and urobilinogen content (data not reported). Except for elevated cholesterol, all hematology, clinical chemistry, and urinalysis parameters in the 50 mg/m^3 group were comparable to controls.

Absolute and relative weights of selected organs are shown in Table 15 (DuPont Co., 1994). Absolute and relative spleen weights were increased and absolute thymus weights were decreased in the 510 and 1120 mg/m^3 groups, compared to control. Relative, but not absolute lung weights were increased compared to control in the 1120 mg/m^3 group. No changes in organ weights of rats treated with 50 mg/m^3 were observed compared to control. Gross pathological examination showed concentration-dependent darkening and enlargement of the spleen in the 510 and 1120 mg/m^3 groups, but not the 50 mg/m^3 group (DuPont Co., 1994). Treatment-related findings on gross and histopathological examination are shown in Table 17. Congestion, hemosiderosis, hematopoiesis and lymphoid cell atrophy of the spleen and lymphoid cell atrophy of the thymus were observed in the 510 and 1120 mg/m^3 groups. No treatment-related histopathological findings of the respiratory tract were observed.

Table 15. Body and Organ Weights of Male Rats Exposed to 4-Nitroaniline by Inhalation for 2 Weeks[a]

Parameter	Exposure Group (mg/m^3)			
	0	**50**	**510**	**1120**
Mean body weight (g)	293.6[b]	298.8 (102)	282.2 (96)	272.0 (93)[d]
Absolute spleen weight (g)	0.616	0.562 (91)	0.950 (154)[d]	1.358 (220)[d]
Relative spleen weight ([organ wt/body wt] × 100)	0.229	0.206 (90)	0.363 (159)[d]	0.550 (240)[d]
Absolute thymus weight (g)	0.586	0.530 (90)	0.468 (80)[c]	0.412 (70)[d]
Relative thymus weight ([organ wt/body wt] × 100)	0.220	0.193 (88)	0.179 (81)	0.167 (76)
Absolute lung weight (g)	1.530	1.498 (98)	1.616 (106)	1.634 (107)
Relative lung weight ([organ wt/body wt] × 100)	0.569	0.551 (97)	0.618 (109)	0.665 (117)[d]

[a]DuPont Co., 1994
[b]Means, () = percent of control
[c]Significantly different from control ($p \leq 0.05$), by least significant difference test
[d]Significantly different from control ($p \leq 0.05$), by least significant difference and Dunnett's tests

Table 16. Selected Hematology and Clinical Chemistry Parameters in Male Rats Exposed to Inhaled 4-Nitroaniline for 2 Weeks[a]

Parameter	Exposure Group (mg/m^3)			
	0	50	510	1120
Observation at End of 2-Week Treatment Period				
Methemoglobin (mg/L)	1.3[b]	2.9 (223)[c]	4.8 (308)[c]	7.0 (538)[c]
Hemoglobin (mg/L)	13.8	13.8 (100)	12.5 (91)[c]	12.5 (91)[c]
RBC ($10^6/\mu L$)	6.86 ± 0.44	6.81 ± 0.21 (99)	6.50 ± 0.33 (95)[c]	5.42 ± 0.44 (79)[c]
MCV (fL)	58 ± 2	57 ± 1 (98)	60 ± 2 (103)	70 ± 4 (121)[c]
MCH (ρg)	22 ± 1	22 ± 1 (100)	22 ± 1 (100)	27 ± 1 (123)[c]
MCHC (g/dL)	37 ± 1	37 ± 1 (100)	37 ± 1 (100) 37 ± 1	39 ± 1 (105)[c]
WBC ($10^3/\mu L$)	9.6 ± 2.7	12.0 ± 2.6 (125)	14.4 ± 3.3 (150)[c]	18.1 ± 4.0 (189)[c]
Cholesterol (mg %)	58 ± 9	69 ± 9 (119)[c]	68 ± 10 (117)[c]	77 ± 10 (133)[c]
Observation at End of 14-Day Recovery Period				
Methemoglobin (mg/L)	0.4	1.0 (250)	1.1 (275)	1.4 (350)[d]
RBC ($10^6/\mu L$)	7.06 ± 0.39	6.61 ± 0.20 (94)	7.00 ± (99)	6.31 ± 0.24 (89)[c]
MCV (fL)	58 ± 1	56 ± 3 (97)	62 ± 1 (107)[c]	64 ± 2 (110)[c]
MCH (ρg)	21 ± 1	22 ± 1 (105)	24 ± 2 (114)[c]	25 ± 1 (119)[c]

[a]DuPont Co., 1994
Means, or means ± SD, () = percent of control
[b]Significantly different from control ($p \leq 0.05$), by least significant difference test
[c]Significantly different from control ($p \leq 0.05$), by least significant difference and Dunnett's tests

Table 17. Incidence of Non-Neoplastic Lesions of the Spleen and Thymus Following Inhalation Exposure of Male Rats to 4-Nitroaniline (2-Week Exposure)[a]

Lesion Type	Exposure Group (mg/m^3)			
	0	50	510	1120
Observation at End of Treatment Period				
Spleen				
Lymphoid cell atrophy	0/5[b]	0/5	5/5	4/5
Congestion	0/5	0/5	5/5	5/5
Focal hematopoiesis	0/5	0/5	5/5	4/5
Hemosiderosis	0/5	0/5	5/5	5/5
Thymus				
Lymphoid cell atrophy	0/5	0/5	5/5	3/5
Observation at End of Recovery Period				
Spleen				
Lymphoid cell atrophy	0/5[c]	0/5	0/5	0/5
Congestion	0/5	0/5	0/5	0/5
Focal hematopoiesis	0/5	0/5	4/5	5/5
Hemosiderosis	0/5	0/5	4/5	5/5
Thymus				
Lymphoid cell atrophy	0/5	0/5	0/5	0/5

[a]DuPont Co., 1994
[b]Number of rats with finding/number of rats examined
[c]Although the methods section of DuPont Co. (1994) states that 6 rats/group were examined for non-neoplastic lesions, the results section reported data for 5 rats/group.

At the end of the 14-day recovery period, no treatment-related differences were observed for body weight or absolute or relative organ weights. MCV and MCHC were elevated in the 510 and 1120 mg/m^3 groups, while MetHb was increased and RBC count was decreased in the 1120 mg/m^3 group. All other hematological, clinical chemistry, and urinalysis parameters affected by the 10-day exposure period were similar to controls after the 14-day recovery period. Hemosiderosis and hematopoiesis persisted through the end of the recovery period (see Table 17), while other splenic and thymic effects were not evident. Results suggest that some effects of inhalation exposure to 4-nitroaniline were reversible. The authors identified 50 and 510 mg/m^3, as the NOAEL and LOAEL values, respectively, based on several findings indicative of hemolytic anemia, methemoglobinemia, and compensatory hematopoietic and leukopoietic responses. However, methemoglobin was significantly increased to 223% of control values in rats exposed to 50 mg/m^3. Although no other abnormal findings consistent with hemolytic anemia or methemoglobinemia were observed, 50 mg/m^3 could be considered a LOAEL for 2-week inhalation exposure of rats to 4-nitroaniline. Assuming the MMAD and geometric standard deviations were the same between 50 mg/m^3 and 510 mg/m^3, the corresponding human equivalent concentration for systemic toxicity was 23 mg/m^3.

Chronic Studies—No studies were located regarding the effects of chronic inhalation exposure of animals to 4-nitroaniline.

Developmental and Reproduction Studies—No studies were located regarding the effects of inhaled 4-nitroaniline on reproduction and fetal development.

Other Studies

Toxicokinetics Studies

Little information on the toxicokinetics of 4-nitroaniline is available (Monsanto Co., 1980c; Chopade and Matthews, 1984). Results of available studies indicate that 4-nitroaniline is well absorbed from the gastrointestinal tract. 4-Nitroaniline undergoes rapid distribution to tissues and does not appear to concentrate in any particular tissue. Elimination of 4-nitronaniline is rapid, predominantly by metabolism and excretion of metabolites into urine.

The absorption, distribution, and elimination of orally administered ^{14}C-4-nitroaniline in rats were studied by Monsanto Co. (1980c). Sprague-Dawley rats (3 males and 3 females) were administered 1.8 mg of 4-nitroaniline in water by gavage. Urine, feces, and expired air were collected for 72 hours. Animals were sacrificed at 72 hours and selected tissues were collected. Within 72 hours after administration, 89.2 to 96.0% of the administered ^{14}C was eliminated in the urine, with approximately 75 to 85% eliminated during the first 8 hours. Fecal elimination accounted for approximately 3.8 to 5.8% and expired air accounted for only 0.01 to 0.07% of administered dose. No data were reported on distribution of ^{14}C remaining in tissues at study completion.

Chopade and Matthews (1984) studied the toxicokinetics of oral 4-nitroaniline in Fisher F/344 rats. Rats (number not reported) were administered 2 or 100 µmol/kg of ^{14}C-4-nitroaniline (equivalent to approximately 0.3 or 13.8 mg/kg) in corn oil. Urine and feces were collected for 72 hours. Animals were sacrificed at 72 hours and tissues (blood, liver, muscle, adipose, kidney, skin, heart, lungs, brain, spleen, bladder, testes, stomach, and small and large intestines) were

analyzed for ^{14}C. Approximately 75−81% of the administered dose was excreted in urine and 13% in feces within 72 hours. Tissue samples contained very low amounts (0.1 to 0.36%) of the administered ^{14}C.

Although studies on the initial tissue distribution of 4-nitroaniline following oral administration were not identified, Chopade and Matthews (1984) studied tissue distribution of ^{14}C following i.v. administration of 10 µmol/kg of ^{14}C-4-nitroaniline (equivalent to approximately 1.38 mg/kg) to Fisher F/344 rats. Animals were sacrificed at 15 and 45 minutes and 2, 7, 24, and 72 hours after administration, and ^{14}C content of tissues was determined for blood, liver, muscle, adipose, kidney, skin, heart, lungs, brain, spleen, bladder, testes, stomach, and small and large intestines. The maximum amount of ^{14}C was observed within 15 minutes of administration, ranging from 3.6% of the administered dose in kidney to 30.6% in muscle. At the 24-hour time point, tissue samples contained only very low amounts (0.06 to 0.72%) of the administered ^{14}C. Analysis of urine at 72 hours identified 9 metabolites of 4-nitroaniline, with 56% of the urinary ^{14}C in the form of two sulfate conjugates. Only 3.5% of the urinary ^{14}C was identified as the parent compound. The elimination half-life of 4-nitroaniline was calculated to be approximately 1 hour.

Genotoxicity Studies

The genotoxicity of 4-nitroaniline has been tested in numerous studies using *in vitro* test systems. These test results generally indicate that 4-nitroaniline has mutagenic and clastogenic activity. Studies investigating the genotoxic potential of 4-nitroaniline *in vivo* were not identified.

4-Nitroaniline has been tested extensively for reverse mutation in *Salmonella typhimurium* (Ames assay) with and without metabolic activation. 4-Nitroaniline was mutagenic in strain TA1538 with, but not without, activation (Garner and Nutman, 1977; Thompson et al., 1983). Although studies reported positive results in strain TA98 with metabolic activation, results without activation have been variable (Chiu et al., 1978; Inoue et al., 1981; Haworth et al., 1983; Thompson et al., 1983; Shahin, 1985; Kawai et al., 1987; Chung et al., 1996; Abmann et al., 1997). With or without S9, 4-nitroaniline gave negative results in strains TA97, TA100, TA1535, and TA1537 (Chiu et al., 1978; Inoue et al., 1981; Haworth et al., 1983; Shahin, 1985; Pai et al., 1985; Kawai et al., 1987; Abmann et al., 1997). Negative results were also reported for the nitroreductase-deficient derivatives of TA98, TA100, and TA1538 (TA98NR, TA100NR, and TA1538NR) (Corbett et al., 1985; Chung et al., 1996). These results suggest that metabolic activation is required for genotoxicity of 4-nitroaniline in bacteria.

In other tests, 4-nitroaniline was not mutagenic in a Chinese hamster ovary (CHO) cell forward gene mutation assay (Monsanto Co., 1984) or in the sex-linked recessive lethal mutation assay using *Drosophila melanogaster* larvae (Zimmering et al., 1989). 4-Nitroaniline induced mutations in L5178Y mouse lymphoma cells in the absence, but not in the presence of S9 (NTP, 1993). 4-Nitroaniline did not induce unscheduled DNA synthesis in primary cultures of adult rat hepatocytes (Thompson et al., 1983), but the U.S. EPA (1985) considered this test inconclusive because of its limited dose range. Galloway et al. (1987) and Chung et al. (1996) reported that 4-nitroaniline induced sister chromatid exchanges in CHO cells with and without metabolic activation, although sister chromatid exchanges in CHO cells were only observed with, not without, S9 activation in the NTP (1993) study. Results from the mouse micronucleus assay revealed no evidence of clastogenicity (judged by the frequency of micronucleated

polychromatic erythrocytes) for 4-nitroaniline (Monsanto Co., 1989). 4-Nitroaniline induced chromosomal aberrations in human lymphocytes tested *in vitro* (Huang et al., 1996). The NTP (1993) reported positive results for chromosomal aberration in CHO cells in the presence of S9 and negative or weakly positive results at a high dose in the absence of S9. In cultured human granulocytes activated to undergo a respiratory burst, 4-nitroaniline formed covalent bonds to RNA, but binding to DNA was at the limit of detection (DNA/RNA binding ratio = 1/80) (Corbett and Corbett, 1988).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR 4-NITROANILINE

Studies investigating the effects of oral exposure to 4-nitroaniline in humans were not identified. Subchronic and chronic toxicity studies in rats and mice identified effects on the hematopoietic system, specifically the conversion of hemoglobin to methemoglobin, as the primary effect of repeated oral exposure to 4-nitroaniline. Methemoglobin differs from normal hemoglobin in that the oxygen-carrying ferrous iron of the heme groups is oxidized to ferric iron. Ferric iron cannot bind oxygen, resulting in functional anemia and tissue hypoxia. In addition, ferric iron oxidizes the globin groups of hemoglobin, leading to denatured hemoglobin molecules that precipitate within the erythrocyte to form Heinz bodies. Due to the presence of Heinz bodies and/or precipitated hemoglobin, erythrocytes are prematurely removed from blood by the spleen, resulting in hemolytic anemia (NTP, 1993). As a compensatory response to methemoglobin-induced functional and hemolytic anemia, hematopoiesis is increased.

There is considerable information in the literature concerning management and treatment of methemoglobinemia in humans, while characterizations of normal ranges and of levels at which cyanosis and other clinical symptoms become apparently vary across the available literature, commonly falling in the range of 6−10%. In humans, methemoglobinemia is diagnosed when the percent of methemoglobin is greater than 1.5% of total hemoglobin (equivalent to approximately 10 g/dL), with a normal value ≤2% (Beers and Berkow, 1999). Although methemoglobin is formed spontaneously in healthy individuals, blood methemoglobin levels are maintained at <2% by two enzymes (cytochrome-b3 reductase and NADPH methemoglobin reductase) that reduce methemoglobin to hemoglobin. In the presence of certain oxidizing drugs and chemicals, the rate of formation of methemoglobin may increase up to 1000-fold, overwhelming reductive enzymes and increasing methemoglobin levels. Symptoms resulting from methemoglobinemia are related to blood methemoglobin levels as follows: <10%—no symptoms; 10−20%—skin discoloration (particularly mucus membranes); 20−30%—anxiety, headache, and dyspnea on exertion; 30−50%—fatigue, confusion, dizziness, tachypnea, and palpitations; 50−70%—coma, seizures, arrhythmias, and acidosis; and >70%—death (Denshaw-Burke and Schoffstall, 2006; Rehman, 2001). Unfortunately little information exists concerning the biological significance of particular metHb levels in rodents and what would correspond to humans, at least regarding relative biological significance.

Effects observed in subchronic and chronic animal studies on hematological parameters (decreased Hgb, Hct, and RBC count, and increased MetHb, reticulocytes, and WBC count) and spleen histopathology (congestion, excessive hemosiderin and hematopoiesis and hyperplasia of

reticulo-endothelial cells) are consistent with 4-nitroaniline-induced methemoglobinemia, anemia, and compensatory erythropoiesis. Other targets for 4-nitroaniline toxicity were not identified. NOAEL and LOAEL values from the available studies are shown in Table 18.

Table 18. Summary of Oral Systemic Toxicity Studies for 4-Nitroaniline

Species	Sex	Dose (mg/kg-day)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses	Reference
Mouse	M, F	0, 10, 30, 300, 1000	gavage 14 d 5 d/wk	ND[a]	10 (7)[b]	Increased methemoglobin	NTP, 1993
Mouse	M, F	0, 1, 3, 10, 30, 100	gavage 90 d 5 d/wk	3 (2)	10 (7)	Increased methemoglobin and histological changes in spleen	NTP, 1993
Rat	M, F	0, 3, 10, 30	gavage 90 d 7 d/wk	ND	3 (3)	Increased methemoglobin and histological changes in spleen	Monsanto Co., 1981b
Mouse	M, F	0, 3, 30, 100	gavage 2 yr 5 d/wk	ND	3 (2)	Increased bone marrow hypercellularity and hemosiderosis of the spleen; minimal LOAEL	NTP, 1993
Rat	M, F	0, 0.25, 1.5, 9	gavage 2 yr 7 d/wk	0.25 (0.25)	1.5 (1.5)	Increased methemoglobin, spleen weights and hepatic and splenic hemosiderosis	Nair et al., 1990

[a]ND = not determined
[b]() = dose normalized over a 7-day week

In addition to subchronic and chronic toxicity studies, 4-nitroaniline was tested for developmental toxicity in rabbits and rats and for reproductive toxicity in rats. The compound did not appear to be a developmental toxicant in rabbits, inducing no fetal effects at a dose (124 mg/kg-day) associated with maternal mortality (Monsanto Co., 1982). In rats, an increased incidence of visceral and skeletal malformations occurred in the fetuses of dams treated with 250 mg/kg-day (Monsanto Co., 1980b) and reduced fetal body weight occurred in those treated with 85 mg/kg-day; however, maternal effects also occurred at these doses. No more sensitive responses were observed in both rabbit and rat developmental studies. The 2-generation study in rats revealed no effects on reproduction at doses up to 9.0 mg/kg-day (Nair et al., 1990).

Subchronic RfD

The most sensitive endpoints by subchronic exposure were increased methemoglobin levels and histological changes in the spleen at daily average dose of 3 mg/kg-day and above in rats (Monsanto Co., 1981b). Mice appeared to be less sensitive to the hematopoietic effects of 4-nitroaniline, with effects on methemoglobin levels and splenic histology occurring consistently only at daily average dose of 7.1 mg/kg-day and above.

Benchmark dose (BMD) modeling was performed on the methemoglobin data from the rats. The data are shown in Table 19. Continuous-variable models in the U.S. EPA BMDS (Version 1.4.1) were fit to the data for changes in blood methemoglobin following exposure to 4-nitroaniline for 3 months. Although data are available to suggest methemoglobin levels in blood can produce adverse effects in humans, no corresponding data were available for rodents. Therefore, a default benchmark response of 1 SD above the control mean was used to estimate the benchmark dose, as recommended by U.S. EPA (2000). Details of the analysis are presented in Appendix A.

Table 19. Methemoglobin Levels (%) in Rats Following a 90-Day Oral Exposure of Rats to 4-Nitroaniline[a]

Sex	Daily Exposure (mg/kg-day)			
	0	**3**	**10**	**30**
Male	1.1 ± 0.4[b] (9)	1.8 ± 0.1[c] (10)	3.3 ± 0.3[c] (9)	5.8 ± 0.6[c] (10)
Female	1.0 ± 0.2 (10)	1.7 ± 0.4[c] (10)	2.8 ± 0.3[c] (10)	4.5 ± 0.3[c] (10)

[a]Monsanto Co., 1981b
[b]Means ± SD, () = number of rats evaluated; standard deviations were calculated from the raw data for this review
[c]Significantly different from control ($p \leq 0.01$), Dunnett's test

Adequate fit was achieved for the female methemoglobin data with constant variance polynomial and Hill models. Although male methemoglobin data were comparable to that of females, BMD modeling indicated that the variance in the male data was not homogeneous. Nevertheless, non-constant variance modeling still failed to provide adequate fit to the variance. Because the constant variance models provided adequate fit to the female data, the same models were also used to model the male data in order to support the lower confidence limit (95%) on the benchmark dose (BMDLs) estimated for the female data. The estimated BMD_{1SD} and $BMDL_{1SD}$ from the female rat data were 1.2 mg/kg-day and 0.95 mg/kg-day, respectively, and they were also consistent with the BMD of 1.5 mg/kg-day and BMDL of 1.2 mg/kg-day estimated from male data.

The histological data from the rats were not suited for BMD modeling, as incidence for the most sensitive lesions was 100% at the lowest dose in both species. In the absence of a BMD for these effects, the LOAEL for splenic histology was considered, along with the BMD for methemoglobinemia determined above, as a potential point of departure (POD) for derivation of the subchronic p-RfD. However, because the splenic changes are considered to be secondary to increased methemoglobin levels, the methemoglobin levels were used as the basis for the subchronic p-RfD.

The **subchronic p-RfD of 0.01 mg/kg-day or 1E-02 mg/kg-day** for 4-nitroaniline, based on the $BMDL_{1SD}$ of 0.95 mg/kg in female rats (Monsanto Co., 1981b), is derived as follows:

Subchronic p-RfD = $BMDL_{1SD} \div UF$
= 0.95 mg/kg ÷ 100
= **0.01 mg/kg-day or 1E-02 mg/kg-day**

The composite uncertainty factor (UF) of 100 is composed of the following:

- A full 10-fold UF for intraspecies differences is used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing anemia or hematopoietic disorders may be more susceptible to oral 4-nitroaniline.
- A full 10-fold UF for interspecies extrapolation is applied to account for potential toxicokinetic and toxicodynamic differences between rats and humans. Methemoglobin reductase activity in rodents has been reported to be approximately 5 to 9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to 4-nitroaniline-induced methemoglobinemia than rodents.
- No UF for database deficiencies is applied because the available database includes well designed subchronic and chronic studies in two species, developmental studies in two species, and a multi-generation reproduction study.

Confidence in the key study is medium to high. Monsanto Co. (1981b) assessed comprehensive endpoints in an appropriate number of animals. The study included multiple effect levels, but a NOAEL was not identified. The key study is supported by a high quality subchronic study in mice conducted by NTP (1993). The database includes subchronic and chronic toxicity studies in two species (rats and mice), developmental toxicity studies in two species (rabbits and rats), and a 2-generation reproduction study; thus, confidence in the database is high. The overall confidence in the subchronic p-RfD for 4-nitroaniline is high.

Chronic RfD

The most sensitive endpoints in the chronic studies are increases in methemoglobin, spleen weights, and hemosiderosis in the liver and spleen. On the basis of these endpoints, the rat study (Nair et al., 1990) identifies a NOAEL of 0.25 mg/kg-day and a LOAEL of 1.5 mg/kg-day and the NTP (1993) mouse study identified a minimal LOAEL of 3 mg/kg-day. Increases in methemoglobin were detected only at a higher dose (30 mg/kg-day) in the mouse study.

As the most sensitive endpoints, the data for increases in methemoglobin and hemosiderosis in the spleen from treated rats (Nair et al., 1990) were considered as potential critical effects for BMD modeling. Because there was significantly high background (62−83%) hemosiderosis in the spleen in the male and female control group, the adversity of this endpoint due to exposure to 4-nitroaniline was not clear. In viewing the hemosiderosis in the spleen as a secondary effect to methemoglobin, only methemoglobin data were modeled for BMD estimation. Because male rats exhibited more sensitivity compared to the females, the $BMDL_{1SD}$ of 0.37 mg/kg-day estimated from the male data (see Appendix A) was used as a POD for derivation of the chronic p-RfD.

The **chronic p-RfD of 0.004 mg/kg-day or 4E-03 mg/kg-day** for 4-nitroaniline, based on the $BMDL_{1SD}$ of 0.37 mg/kg-day in rats (Nair et al., 1990), is derived as follows:

Chronic p-RfD = $BMDL_{1SD} \div UF$
= 0.37 mg/kg-day ÷ 100
= **0.004 mg/kg-day or 4E-03 mg/kg-day**

The composite UF of 100 is composed of the following:

- A full 10-fold UF for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans. Individuals with pre-existing anemia or hematopoietic disorders may be more susceptible to oral 4-nitroaniline.
- A full 10-fold UF for interspecies extrapolation is applied to account for potential toxicokinetic and toxicodynamic differences between rats and humans. Methemoglobin reductase activity in rodents has been reported to be approximately 5 to 9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to 4-nitroaniline-induced methemoglobinemia than rodents.
- No UF for database deficiencies was applied. Well designed subchronic and chronic studies in two species are available, as well as developmental studies in two species and a multi-generation reproduction study.

Confidence in the key study is high. Nair et al. (1990) assessed comprehensive endpoints in an appropriate number of animals. The study includes multiple dose levels. The key study is supported by a high quality chronic study in mice conducted by NTP (1993). The database includes subchronic and chronic toxicity studies in two species (rats and mice), developmental toxicity studies in two species (rabbits and rats), and a 2-generation reproduction study; thus, confidence in the database is high. The overall confidence in the chronic p-RfD for 4-nitroaniline is high.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfCs FOR 4-NITROANILINE

Limited information is available regarding the effects of 4-nitroaniline by the inhalation route of exposure in humans or animals. Humans who inhaled powdered 4-nitroaniline (dermal exposure also occurred) over an 8-hour period experienced signs and symptoms consistent with increased methemoglobin formation (Anderson, 1946); however, exposure was not quantified and hematological parameters were not assessed. Chronic inhalation studies of 4-nitroaniline in animals were not identified. Short-term (e.g., 2 to 4 weeks) toxicity studies in rats identified effects on the hematopoietic system, specifically the conversion of hemoglobin to methemoglobin, as the primary effect of inhalation exposure to 4-nitroaniline (Nair et al., 1986; DuPont Co., 1994). Results are consistent with 4-nitroaniline-induced methemoglobinemia, followed by anemia and compensatory erythropoiesis, as discussed above for derivation of subchronic and chronic p-RfDs. Male and female rats exposed 6 hours/day, 5 days/week (whole body exposure), for 4 weeks, to 10−80 mg/m^3 of an aerosol of 4-nitroaniline showed a dose-related increase in blood methemoglobin levels and an increased incidence of morphological changes in the red blood cells consisting of polychromasia (males and females) and anisocytosis (females only) (Nair et al., 1986). Nair et al. (1986) examined several additional endpoints including clinical chemistry and gross and microscopic appearance of tissues and organs (upper and lower respiratory tract included); the only treatment-related findings were dose-related increases in severity of splenic hemosiderosis and extramedullary hematopoiesis. The low exposure level of 10 mg/m^3 (HEC = 4.2 mg/m^3) was identified as a LOAEL in this study. Similar results were observed in male rats exposed (head only) to inhaled

4-nitroaniline at concentrations of 50, 510, and 1120 mg/m^3 for 2 weeks, with significantly elevated methemoglobin observed in all treatment groups (DuPont Co., 1994). Compensatory hematopoietic effects were observed in the 510 and 1120 mg/m^3 treatment groups. Based on increased methemoglobin levels, a LOAEL of 50 mg/m^3 (HEC = 23 mg/m^3) is identified; a NOAEL is not established.

Based on the results of available inhalation studies, methemoglobinemia and related hematopoietic effects are identified as the critical effects for derivation of subchronic and chronic p-RfCs. These are also the critical effects in rats and mice exposed orally to 4-nitroaniline (NTP, 1993; Nair et al., 1990; Monsanto Co., 1981b). Additional information on the clinical significance of methemoglobinemia and compensatory effects was discussed above for derivation of subchronic and chronic p-RfDs. Both short-term inhalation studies (Nair et al., 1986; DuPont Co., 1994) were conducted in an adequate number of animals and examined appropriate endpoints. However, the study by DuPont Co. (1994) exposed animals for only 2 weeks and identified a higher LOAEL than the study by Nair et al. (1986). Thus, the 4-week inhalation study in male and female rats (Nair et al., 1986) is selected as the critical study for derivation of the subchronic and chronic p-RfCs.

Subchronic RfC

To determine the POD for derivation of the subchronic p-RfC, exposure concentrations were first adjusted for continuous exposure ($Conc_{[ADJ]}$), as shown in Table 20, and then followed by human equivalent concentration (HEC) conversions ($Conc_{[HEC]}$) based on $Conc_{[ADJ]}$ (calculated for extrarespiratory effects [methemoglobinemia]) using the regional deposited dose ratio (RDDR) computer program, as specified in the RfC guidelines (U.S. EPA, 1994b) (see Table 20). Because Nair et al. (1986) report body weight as 204−243 g for both male and female rats, an average of 223.5 g body weight was used in the calculation of the RDDR. The reported average particle sizes (MMAD ± GSD) of 0.8 ± 5.42, 1.37 ± 4.04 and 0.78 ± 6.42 μm were used for the 1.8, 5.7, and 14 mg/m^3 groups, respectively (Nair et al., 1986).

Table 20. Concentration-Response Data for 4-Nitroaniline-induced Methemoglobinemia (with Concentrations Expressed in Terms of HEC for Systemic Effects) in Male and Female Rats Exposed by Inhalation for 4 Weeks[a]

Sex	Conc (mg/m^3)	$Conc_{[ADJ]}$[b] (mg/m^3)	RDDR	$Conc_{[HEC]}$[c] (mg/m^3)	MetHb (% of total Hb)
Male[d]	0	0	—	0	1.5 ± 0.8[e]
	10	1.8	2.347	4.2	2.8 ± 1.4[g]
	32	5.7	2.528	14	3.6 ± 1.1[f]
	80	14	2.313	33	5.5 ± 2.1[f]
Female[d]	0	0	—	0	1.4 ± 1.0
	10	1.8	2.347	4.2	1.4 ± 1.1
	32	5.7	2.528	14	3.1 ± 1.4[f]
	80	14	2.313	33	3.8 ± 1.3[f]

[a]Nair et al., 1986
[b]$Conc_{[ADJ]}$ = Conc × 6/24 hours × 5/7 days
[c]$Conc_{[HEC]}$ = $Conc_{[ADJ]}$ × RDDR
[d]10 rats per treatment group
[e]Mean ± SD
[f]Significantly different from control ($p \leq 0.01$), as reported by the researchers
[g]Not listed as statistically significant in the report, but significantly different from control ($p = 0.02$) by unpaired t-test (two tailed) performed for this review
—: RDDR not determined for control group

To determine the POD for derivation of the subchronic p-RfD, benchmark concentration (BMC) modeling of the methemoglobin data was conducted using the U.S. EPA BMDS (Version 1.4.1). As recommended by U.S. EPA (2000), 1 SD above the control mean was used as the BMR level. Details of the BMC analysis are presented in Appendix A. Histology data showing increased severity of splenic lesions in treated rats is not suitable for benchmark concentration modeling.

Adequate fit was achieved for the male methemoglobin data, and a BMC_{1SD} of 3.2 mg/m^3 and lower confidence limit (95%) on the benchmark concentration ($BMCL_{1SD}$) of 1.7 mg/m^3 were determined from the male rat data. The female data were best fit with dropping the high concentration group, and the estimated BMC_{1SD} and $BMCL_{1SD}$ were 8.7 mg/m^3 and 5.8 mg/m^3 respectively. The lower $BMCL_{1SD}$ of 1.7 mg/m^3, from the male rats, was selected as the POD for derivation of the subchronic p-RfC for 4-nitroaniline.

The **subchronic p-RfC of 0.02 mg/m^3 or 2E-02 mg/m^3** for 4-nitroaniline, based on the $BMCL_{1SD}$ of 1.7 mg/m^3 in male rats (Nair et al., 1986), was derived as follows:

Subchronic p-RfC = $BMCL_{1SD} \div UF$
= 1.7 $mg/m^3 \div 100$
= **0.02 mg/m^3 or 2E-02 mg/m^3**

The composite UF of 100 is composed of the following:

- A full 10-fold UF for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans. Individuals with pre-existing anemia or hematopoietic disorders may be more susceptible to inhaled 4-nitroaniline.
- A partial 3-fold UF for interspecies extrapolation is applied to account for potential toxicodynamic differences between rats and humans. Methemoglobin reductase activity in rodents has been reported to be approximately 5 to 9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to 4-nitroaniline-induced methemoglobinemia than rodents. However, the impact of this on toxicodynamic differences is uncertain. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for toxicokinetic differences between rats and humans; thus, the full 10-fold UF for interspecies extrapolation is reduced.
- An UF for exposure duration is not included, although exposure is less than subchronic. Based on results of subchronic oral toxicity studies, maximum blood methemoglobin levels appear to be reached within 2 to 7 weeks of exposure to 4-nitroaniline. These levels decline and reach a plateau within 3 months. It is unlikely that the duration-related plateau varies with route of exposure.
- A partial 3-fold UF for database insufficiencies is included. Although the database lacks developmental toxicity and multi-generation reproduction studies for inhaled 4-nitroaniline, high quality developmental toxicity studies and a 2-generation reproduction study for oral exposure are available. The oral studies indicate statistically significant increases in resorptions, internal malformations of the kidney, and external malformations of the tail and digits, and decreases in the percentage of live fetuses. These effects were seen in the high dose (250 mg/kg-day) group only. Because the results of oral exposure studies show 4-nitroaniline-induced developmental effects at a relatively high dose only, a full 10-fold UF for database deficiency is not applied.

Confidence in the key study is medium. Although appropriate endpoints are evaluated in an adequate number of animals, the exposure duration is short and particle size is highly variable. Confidence in the database is medium due to the lack of additional subchronic studies in a second species, the lack of a developmental toxicity study, and the lack of a multi-generation reproductive toxicity study by the inhalation route of exposure. However, the lack of inhalation data is tempered by the availability of high quality oral data supporting the same critical effect. The overall confidence in the subchronic p-RfC is medium.

Chronic RfC

Chronic toxicity studies for inhaled 4-nitroaniline are not available. Therefore, the chronic p-RfC is based on the $BMCL_{1SD}$ of 1.7 mg/m^3 derived for male rats exposed to inhaled 4-nitroaniline for 4-weeks (Nair et al., 1986). The **chronic p-RfC of 0.006 mg/m^3 or 6E-03 mg/m^3** for 4-nitroaniline, based on the $BMCL_{1SD}$ of 1.7 mg/m^3, is derived as follows:

Chronic p-RfC = $BMCL_{1SD} \div UF$
= $1.7\ mg/m^3 \div 300$
= **$0.006\ mg/m^3$ or 6E-03 mg/m^3**

The composite UF of 300 is composed of the following:

- A full 10-fold UF for intraspecies differences is used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing anemia or hematopoietic disorders may be more susceptible to oral 4-nitroaniline.
- A partial 3-fold UF for interspecies extrapolation is used to account for the potential toxicodynamic differences between rats and humans. Methemoglobin reductase activity in rodents has been reported to be approximately 5 to 9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to 4-nitroaniline-induced methemoglobinemia than rodents. However, the impact of this on toxicodynamic differences is uncertain. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for toxicokinetic differences between rats and humans; thus, the full 10-fold UF for interspecies extrapolation is reduced.
- A partial 3-fold UF is applied for a less than chronic exposure duration. Based on results of subchronic oral toxicity studies, maximum blood methemoglobin levels appear to be reached within 2 to 7 weeks of exposure. These values then decline and reach a plateau within 3 months. It is unlikely that the duration-related plateau varies with route of exposure. However, due to lack of chronic inhalation data, it is not known if lifetime inhalation exposure to 4-nitroaniline produces adverse effects in other organs, such as the respiratory tract.
- A partial 3-fold UF for database insufficiencies is used to account for the lack of developmental toxicity and multi-generation reproduction studies for inhaled 4-nitroaniline. Because the results of oral exposure studies show 4-nitroaniline-induced developmental effects at a relatively high dose only, a full 10-fold UF for database deficiency is not applied.
- However, because the results of high quality oral exposure studies show a lack of 4-nitroaniline-induced developmental or reproductive effects, a full 10-fold UF for database deficiencies is not applied.

Confidence in the key study is medium. Although appropriate endpoints are evaluated in an adequate number of animals, the exposure duration is short and particle size is highly variable. Confidence in the database is medium due to the lack of additional subchronic studies in a second species, the lack of chronic studies, the lack of a developmental toxicity study, and the lack of a multi-generation reproductive toxicity study by the inhalation route of exposure. However, the lack of inhalation data is tempered by high quality oral studies identifying the same systemic target. The overall confidence in the chronic p-RfC is medium.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 4-NITROANILINE

Weight-of-Evidence Descriptor

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), the available evidence for oral exposure to 4-nitroaniline is suggestive of carcinogenic potential based on limited (equivocal) evidence of carcinogenicity in male mice in the NTP (1993) gavage bioassay, but no available data in humans. Results of the NTP (1993) bioassay show significant increases over the ranges for historical controls and significant positive trends for vascular tumors in the liver (hemangiosarcomas) and at all sites (hemangiomas and hemangiosarcomas combined) in male mice treated orally for 2 years (see Table 10); thus, the NTP classified evidence for carcinogenesis as "equivocal." In addition, the occurrence of vascular tumors in one male mouse treated with 100 mg/kg for 9 months and in one treated with 30 mg/kg for 15 months strengthens the case for an etiologic connection. The incidence of vascular tumors in female mice exposed to 4-nitroaniline is not significantly different from study or historical controls. Exposure-related tumors have not been observed in male or female rats exposed to oral 4-nitroaniline for 2 years (Nair et al., 1990). Studies evaluating the carcinogenic potential of inhaled 4-nitroaniline in humans or animals were not located.

Mode-of-Action Discussion

The U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment defines mode of action as a sequence of key events and processes starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in cancer formation. Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation and immune suppression (page 1−10).

The mechanism of 4-nitroaniline-induced carcinogenicity has not been determined; however, available evidence suggests that vascular tumors in the liver (hemangiosarcomas) and at all sites (hemangiomas and hemangiosarcomas) observed in mice following oral exposure to 4-nitroaniline may arise from genetic mechanisms. Other potential modes of action for 4-nitroaniline-induced hemangiomas and hemangiosarcomas have not been identified; thus, only a mutagenic mode of action is considered.

Mutagenic Mode of Action

Key Events—Numerous studies using in vitro test systems provide evidence that 4-nitroaniline has mutagenic and clastogenic activity in vitro, although evidence of genotoxic activity in vivo is lacking. In bacteria, 4-nitroaniline induced mutations with metabolic activation, although conflicting results have been reported in the absence of metabolic activators (Abmann et al., 1997; Chiu et al., 1978; Chung et al., 1996; Inoue et al., 1981; Garner and Nutman, 1977; Haworth et al., 1983; Thompson et al., 1983; Shahin, 1985; Kawai et al., 1987). In mammalian cells, 4-nitroaniline-induced sister chromatid exchanges in CHO cells (Chung et al., 1996; Galloway et al., 1987) and chromosomal aberrations in human lymphocytes (Huang et al., 1996) have been documented. The NTP (1993) reported positive results for chromosomal aberrations in cultured CHO cells in the presence of metabolic activation and

negative or weakly positive results in the absence of metabolic activation. Studies evaluating the genotoxicity of 4-nitroaniline in cells of vascular origin or in vivo in humans are lacking.

Strength, Consistency, Specificity of Association—Although NTP (1993) reported equivocal evidence of the potential of oral 4-nitroaniline to induce hemangiomas and hemangiosarcomas in mice, evidence demonstrating that 4-nitroaniline can induce mutagenic changes in vascular cells is lacking. Thus, data are not available to link results of genotoxicity studies to the development of hemangiomas and hemangiosarcomas reported by NTP (1993).

Dose-Response Concordance—A dose-response concordance has not been established between the development of hemangiomas and hemangiosarcomas and mutagenesis, since in vivo evidence of mutagenicity for 4-nitroaniline is not available. Furthermore, no data are available on the mutagenic potential of 4-nitroaniline in vascular cells following in vitro or in vivo exposure.

Temporal Relationships—Hemangiosarcomas of the liver (8%) and combined hemangiomas and hemangiosarcomas at all sites (20%) have been observed in male mice exposed to 4-nitroaniline for 2 years (NTP, 1993). At the 9- and 12-month interim sacrifices, hemangiosarcoma of the liver (1/10) and hemangioma of the urinary bladder (1/10) were observed. However, due to the lack of data on the mutagenic potential of 4-nitroaniline in vascular cells, the temporal relationship between possible mutagenic mechanisms and the development of hemangiomas and hemangiosarcomas could not be assessed.

Biological Plausibility and Coherence—Although several studies provide evidence that 4-nitroaniline has mutagenic and clastogenic activity in vitro, no evidence is available linking mutagenesis in vascular cells to the development of hemangiomas and hemangiosarcomas.

Conclusions—Limited evidence supports the mutagenic mode of action for 4-nitroaniline tumorigenicity. Although in vitro studies provide evidence that 4-nitroaniline is capable of eliciting genotoxic effects in mammalian cells, two key uncertainties remain: (1) data evaluating the genotoxic potential of 4-nitroaniline in vivo are lacking, and (2) no evidence linking mutagenesis to the development of vascular cell tumors is available.

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

The NTP (1993) 2-year carcinogenicity study yielded equivocal evidence in male mice of 4-nitroaniline-induced hemangiosarcoma of the liver and hemangiomas or hemangiosarcomas at all sites. Although there is a significant trend in both data sets, none of the dose groups are statistically different from the concurrent controls. The ***Guidelines for Carcinogen Risk Assessment*** (U.S. EPA, 2005) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." The cancer bioassay for 4-nitroaniline is generally well-conducted and the data from this study are considered adequate to support a quantitative cancer dose-response assessment. In addition, hemangiosarcomas are generally considered rare tumors in both animals and humans.

Considering the data and uncertainty associated with the suggestive nature of the tumorigenic response, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of 4-nitroaniline is deemed appropriate.

The NTP (1993) 2-year carcinogenicity study, yielding equivocal evidence in male mice of 4-nitroaniline-induced hemangiosarcomas of the liver and hemangiomas or hemangiosarcomas at all sites, was used for derivation of the oral slope factor (OSF) for 4-nitroaniline. To determine the POD for derivation of the OSF, BMD modeling was conducted using the U.S. EPA BMDS (Version 1.4.1) (U.S. EPA, 2000). The BMD modeling results are summarized in Appendix A. Adequate model fit was obtained for the hemangiosarcoma (liver) and hemangiomas or hemangiosarcomas (all sites) incidence data using the multistage model. The BMD model results for hemangiomas or hemangiosarcomas (all sites) yielded a $BMDL_{10}$ of 31 mg/kg-day, a value that is lower than the $BMDL_{10}$ for hemangiosarcomas (liver), and it is selected for derivation of the final OSF because it represents a more sensitive indicator of tumorigenicity.

The human equivalent dose (HED) of the $BMDL_{10}$ of 31 mg/kg-day is calculated as follows:

$$\begin{aligned} BMDL_{10\,HED} &= BMDL_{10} \times (BW_{animal} / BW_{human})^{1/4} \\ &= 31 \times (0.0472 / 70)^{1/4} \\ &= 31 \times 0.16 \\ &= 5.0 \text{ mg/kg-day} \end{aligned}$$

where

BW_{human} = 70 kg (human reference body weight)
BW_{animal} = 0.0472 kg (time weighted average body weight for male mice in the NTP study)

In the absence of a known mode of action for carcinogenicity of oral 4-nitroaniline, a linear approach was taken to calculate the OSF (U.S. EPA, 2005). In order to linearly extrapolate cancer risks from the $BMDL_{10\,HED}$ to the origin, a cancer OSF was calculated as the ratio $0.1/BMDL_{10\,HED}$. Taking the $BMDL_{10\,HED}$ of 5.0 mg/kg-day for hemangiomas or hemangiosarcomas (all sites) in male mice as the POD, an **oral slope factor of $0.02\ (mg/kg\text{-}day)^{-1}$** is calculated as follows:

$$\begin{aligned} \textbf{p-OSF} &= 0.1 \div BMDL_{10\,HED} \\ &= 0.1 \div 5.0 \text{ mg/kg-day} \\ &= \textbf{0.02 or 2E-02 } \textbf{(mg/kg-day)}^{-1} \end{aligned}$$

Using this OSF to calculate risks greater than, or approaching 0.01 is inappropriate because of the nature of the OSF derivation, i.e., the dose-response slope is calculated based on the experimental point of departure linearized to the origin (by default). The slope of the line close to and above the POD is not reliable, thus the risk calculated at this point provides too much uncertainty. Therefore, a risk of 0.01 should be considered a maximum risk (U.S. EPA, 1989; RAGS, Part A, Section 8.3.1) for this chemical in this situation.

Estimates of continuous lifetime exposure to 4-nitroaniline that correspond to specified risk levels (i.e., 1×10^{-4}, 1×10^{-5}, 1×10^{-6}) are shown in Table 21.

Table 21. Continuous Lifetime Exposure Estimates Corresponding to Specified Cancer Risk for 4-Nitroaniline

Risk[a]	Dose
1×10^{-4} Risk	5×10^{-3} mg/kg-day
1×10^{-5} Risk	5×10^{-4} mg/kg-day
1×10^{-6} Risk	5×10^{-5} mg/kg-day

[a]Extra risk due to 4-nitroaniline exposure

Inhalation Exposure

No human or animal studies examining the carcinogenicity of 4-nitroaniline following inhalation exposure have been located. Therefore, derivation of an inhalation unit risk is precluded.

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APPENDIX A. DETAILS OF BMD ANALYSIS FOR 4-NITROANILINE

The model fitting procedure for continuous data is as follows. The BMD modeling has been conducted with the U.S. EPA's BMD software (BMDS Version 1.4.1). For continuous data set, the original data were modeled with all the continuous models available within the software. An adequate fit was judged based on (1) the goodness of fit *p*-value, (2) the scaled residue at the range of benchmark response (BMR), and (3) the visual inspection of the model fit. Among all the models that provide adequate data fit, the lowest BMDL is selected if the BMDLs estimated from different models varied >3-fold. Otherwise, the BMDL from the model with the lowest AIC is selected as the POD. In addition to the three criteria for judging the adequate model fit, whether the variance needed to be modeled, and if so, how it was modeled also determines the final use of model results. If a homogenous variance model is recommended based on statistics provided from the BMD model runs, the final BMD results would be estimated from a homogenous variance model. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling.

Subchronic RfD

Following the above procedure, continuous-variable models in the U.S. EPA BMDS (Version 1.4.1) were fit to the data shown in Table 19 for blood methemoglobin in male and female rats. The variance data for male rats were not adequately fit by assuming constant variance or by applying the nonhomogenous variance model in the BMDS, either with all doses included or with the high dose dropped. Thus, data sets for male rats might be considered not suitable for BMD modeling (see Table A-1). However, based on variance homogeneity test (Test 2) in female BMD model runs, a constant variance model is recommended for the female methemoglobin data. For reference purpose, here we also present the BMD model results for the male methemoglobin data with constant variance model settings.

Table A-1. Model Predictions for Changes in Methemoglobin Levels (% of Total Hemoglobin) in Male Rats Exposed to Oral 4-Nitroaniline for 12 Weeks[a]

Model	Homogeneity Variance *p*-value[b]	Goodness of fit *p*-value[b]	AIC for fitted model	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear	<0.0001	0.0010	-16.80	3.0	2.5
Polynomial	**<0.0001**	**0.8607**	**-28.61**	**1.5**	**1.2**
Power	<0.0001	0.0010	-16.80	3.0	2.5
Hill	<0.0001	N/A	-26.64	1.7	1.1

[a]Monsanto Co., 1981b
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose

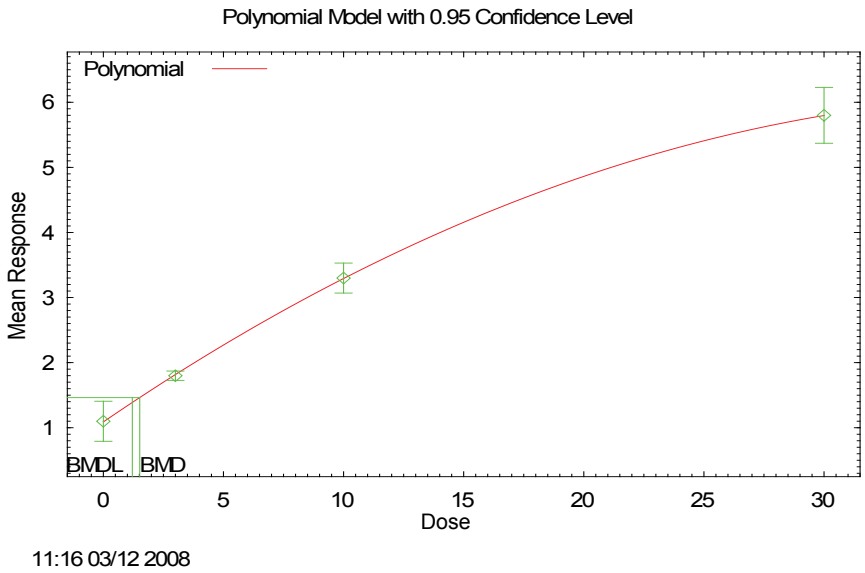

Figure A-1. Polynomial BMD model for male methemoglobin data (Monsanto Co., 1981b)

With all doses included, the variance data for female rats were fit by the constant variance model as suggested by the Test 2 for the homogeneity variance ($p = 0.2107$) in each model run. With the homogeneous variance model applied, the polynomial (2nd degree) and Hill models provide adequate fit to the data while the linear and power model do not, as shown in Table A-2 and Figure A-2. Because the estimated BMDLs from the polynomial and Hill models differed by less than 3-fold, the Hill model, which provides a lower AIC value, is considered a better model for this data set. Therefore, the BMDs and the 95% lower confidence limits (BMDLs) associated with a change of 1 standard deviation (SD) from the control are calculated using the Hill model with homogenous variance model applied (shown in Table A-2 and Figure A-2), and the estimated $BMDL_{1SD}$ is 0.95 mg/kg-day for female rats. This estimated BMDL was also consistent with the BMDL estimated from male data shown in Table A-1, and Figure A-1 (even though constant variance models might not be adequate in modeling the male hemoglobin data); therefore, this BMDL was used as the POD in deriving subchronic p-RfD.

Table A-2. Model Predictions for Changes in Methemoglobin Levels (% of Total Hemoglobin) in Female Rats Exposed to Oral 4-Nitroaniline for 12 Weeks[a]

Model	Variance model *p*-value	Goodness of fit *p*-value[b]	AIC for fitted model	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear (constant variance)	0.2107	<.0001	-29.53	3.5	2.9
Polynomial (2-degree)[c,d] (constant variance)	0.2107	0.4204	-49.72	1.4	1.2
Power[e] (constant variance)	0.2107	<.00001	-29.53	3.5	2.9
Hill[e] (constant variance)	**0.2107**	**0.7797**	**-50.29**	**1.2**	**0.95**

[a]Monsanto Co., 1981b
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0
[d]Insufficient degrees of freedom to fit higher degree polynomials
[e]Power restricted to ≥1

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output)

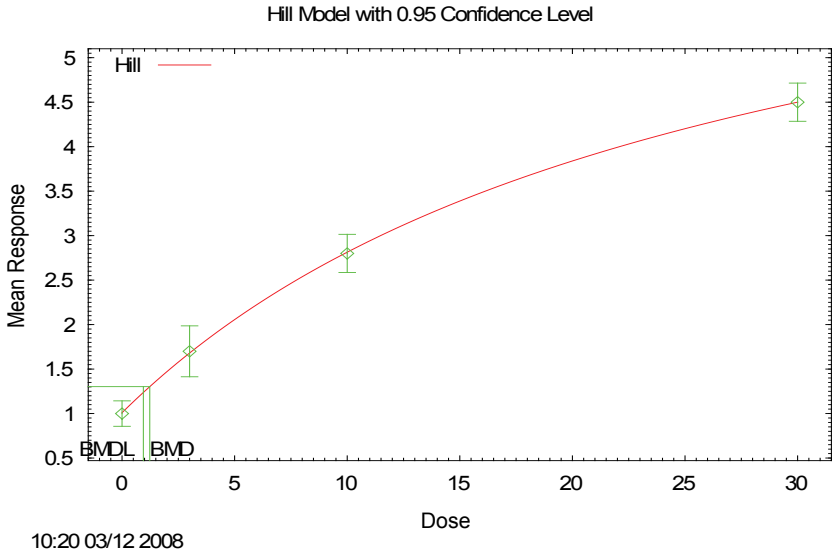

Figure A-2. Hill BMD model for female methemoglobin data (Monsanto Co., 1981b)

Chronic p-RfD

The spleen hemosiderosis data (see Table 12) observed in treated rats (Nair et al., 1990) are modeled with BMD model. Only the responses observed in male rats are modeled because the males had more incidences than the corresponding females in the mid-dose group. All the available continuous-variable models in the U.S. EPA BMDS (Version 1.4.1) are fit to the data shown in Table 11. The test for homogeneity of variance indicated that a nonhomogenous variance model is appropriate (Test 2, $p < 0.0001$). Therefore, all the formal BMD model runs are conducted with nonhomogenous models. As discussed in the main document, the BMR is set to 1.0 standard deviation. The BMD modeling results are summarized in the Table A-3 and Figure A-3. Adequate model fit to the data was obtained only from nonhomogenous polynomial model with a goodness of fit *p*-value of 0.2687 (see Table A-3 and Figure A-3). The resulted BMD and BMDL are 0.53 and 0.37 mg/kg-day, respectively.

Table A-3. Model Predictions for Changes in Methemoglobin Levels in Male Rats Exposed to Oral 4-Nitroaniline for 2 Years[a]

Model	Variance model *p*-value[b]	Goodness of fit *p*-value[b]	AIC for fitted model	BMD_{1sd} (mg/kg-day)	$BMDL_{1sd}$ (mg/kg-day)
Linear	1.0	0.0475	-78.88	0.89	0.69
Polynomial	**1.0**	**0.2687**	**-81.75**	**0.53**	**0.37**
Power	1.0	0.0475	-78.88	0.89	0.69
Hill	1.0	<0.0001	-54.97	5.6	N/A

[a]Nair et al., 1990
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose

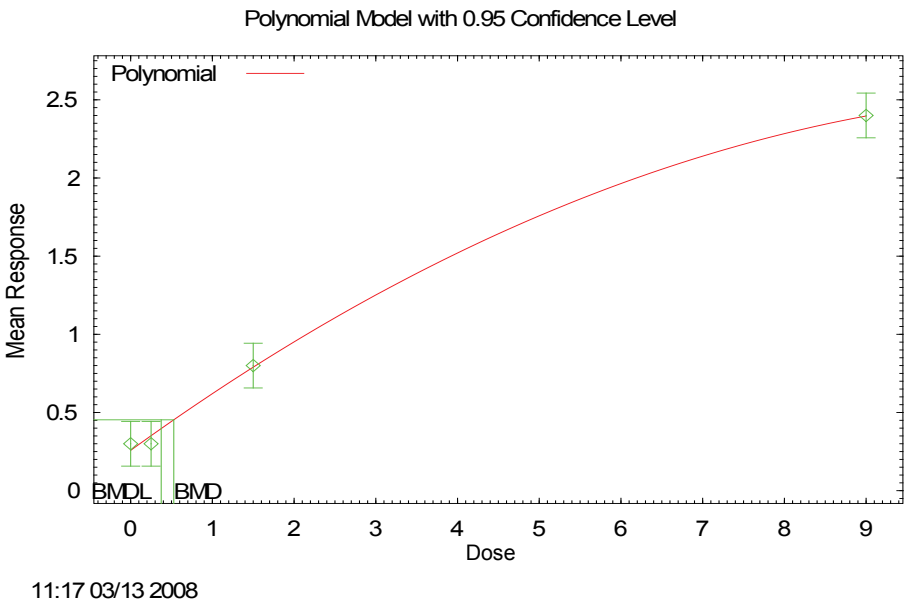

Figure A-3. Polynomial BMD model for male methemoglobin data (Nair et al., 1990)

Subchronic RfC

Following the above procedure, continuous-variable models in the U.S. EPA BMDS (Version 1.4.1) have been fit to the data shown in Table 20 for blood methemoglobin in male and female rats. Summary statistics and output for benchmark concentration (BMC) modeling of the 4-week inhalation data are provided in Table A-4. Data for male rats are modeled with all concentration groups included. Among all the models available, adequate fit ($p = 0.1026$) to methemoglobin means in male rats was only obtained with nonconstant variance Hill model, and all the other nonconstant variance models failed to model the mean responses. All the constant variance models failed to model the variance in the data (see Table A-4 and Figure A-4). When the high concentration group was removed from the data set for BMD modeling, the data set could be modeled successfully with constant variance models; however, no significant improvement in the goodness-of-fit *p*-values was achieved (see Table A-4). Thus, the estimated $BMCL_{1SD}$ of 1.7 mg/m^3 (HEC) based on the Hill model for the full data set is considered more appropriate for male rats because it employed all the data points. For female rats, adequate fit was obtained with three constant variance models (Linear, Polynomial, and Power) for the all concentrations groups. However, improved fit to methemoglobin means was obtained with a constant variance linear model ($p = 0.2564$) after the high concentration group was removed from analysis (see Table A-4 and Figure A-5). The estimated $BMCL_{1SD}$ was 5.8 mg/m^3 (HEC) for female rats. Nonconstant variance models were not used to model the female because constant variance models provided adequate fit to the data. Compared to the female data, modeling of male rat data resulted in a lower $BMCL_{1SD}$ of 1.7 mg/m^3.

Table A-4. Model Predictions for Changes in Methemoglobin Levels (% of Total Hemoglobin) in Male and Female Rats Exposed to 4-Nitroaniline by Inhalation for 4 Weeks[a]

Model	Variance model *p*-value[b]	Goodness of fit *p*-value[b]	AIC for fitted model	BMC_{1SD} (mg/m^3)	$BMCL_{1SD}$ (mg/m^3)
Males—All concentrations					
Linear (nonconstant variance)	0.4431	0.0894	69.25	8.2	5.4
Polynomial[d,e] (nonconstant variance)	0.4431	0.0615	69.92	4.9	2.5
Power[e] (nonconstant variance)	0.4431	0.0894	69.25	8.2	5.4
Hill[e] (nonconstant variance)	**0.4431**	**0.1026**	**69.09**	**3.2**	**1.7**
Males—High concentration dropped					
Linear (constant variance)	0.2234	0.1223	42.40	8.1	5.5
Polynomial[d,e] (constant variance)	0.2234	0.1223	42.40	8.1	5.5
Power[e] (constant variance)	0.2234	0.1223	42.40	8.1	5.5
Hill[e] (constant variance)	N/A	N/A	N/A	N/A	N/A
Females—All					
Linear (constant variance)	0.7076	0.1609	60.71	15	11
Polynomial[d,e] (constant variance)	0.7076	0.1829	60.83	8.5	5.0
Power[e] (constant variance)	0.7076	0.1609	60.71	15	11
Hill[e] (constant variance)	0.7076	N/A	61.06	13	5.6
Females—High concentration dropped					
Linear (constant variance)	**0.537**	**0.2564**	**44.01**	**8.7**	**5.8**
Polynomial[d,e] (constant variance)	0.537	N/A	44.72	12	5.9
Power[e] (constant variance)	0.537	N/A	44.72	13	6.4

Table A-4. Model Predictions for Changes in Methemoglobin Levels (% of Total Hemoglobin) in Male and Female Rats Exposed to 4-Nitroaniline by Inhalation for 4 Weeks[a]

Model	Variance model *p*-value[b]	Goodness of fit *p*-value[b]	AIC for fitted model	BMC_{1SD} (mg/m^3)	$BMCL_{1SD}$ (mg/m^3)
Hill[e] (constant variance)	N/A	N/A	N/A	N/A	N/A

[a]Nair et al., 1986
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0
[d]Insufficient degrees of freedom to fit higher degree polynomials
[e]Power restricted to ≥1

AIC = Akaike's Information Criteria; BMC = benchmark concentration; BMCL = lower confidence limit (95%) on the benchmark concentration; NA = not available (BMD software could not generate a model output)

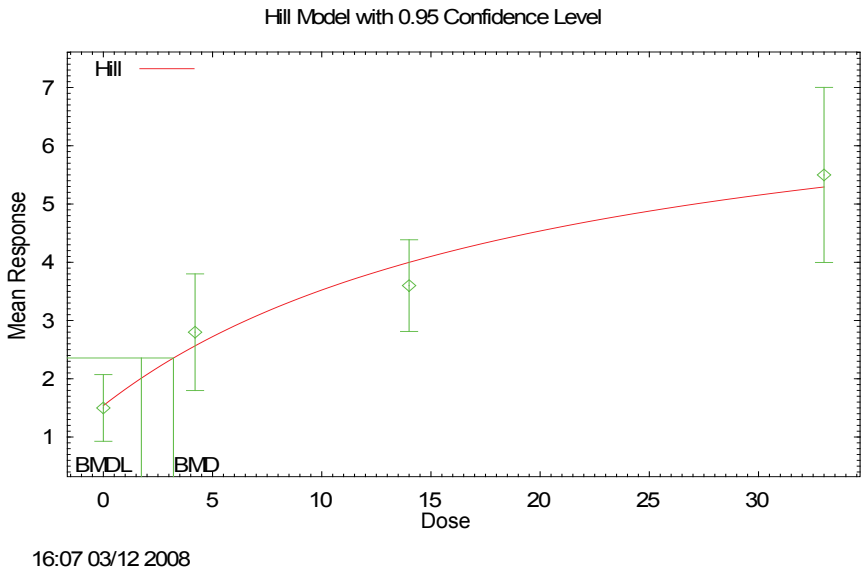

Figure A-4. Hill BMC model for male methemoglobin data (Nair et al., 1986)

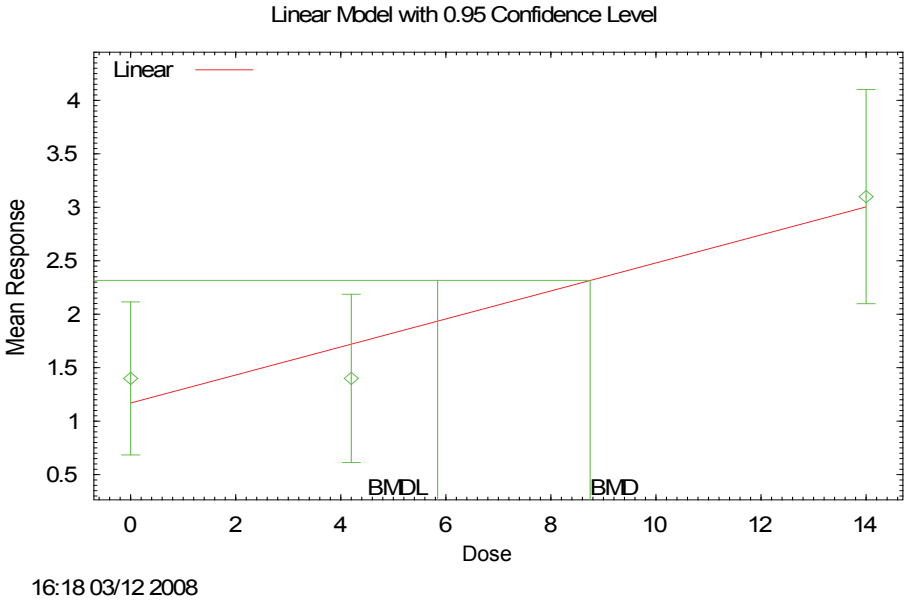

Figure A-5. Linear BMC model for female methemoglobin data without the high concentration (Nair et al., 1986)

Oral Cancer Slope Factor

The NTP (1993) 2-year carcinogenicity study, yielding equivocal evidence in male mice of 4-nitroaniline-induced hemangiosarcomas of the liver and hemangiomas or hemangiosarcomas at all sites, was used for derivation of the oral slope factor (OSF) for 4-nitroaniline (see Table A-5). To determine the POD for derivation of the OSF, BMD modeling was conducted using the U.S. EPA BMDS (Version 1.4.1) (U.S. EPA, 2000). Predicted doses associated with a 10% extra risk (BMD10) were calculated, with the BMDL10 represented by the 95% lower confidence limit on the BMD10. Multistage models were fit to the incidence data for tumors (hemangiosarcomas of the liver and hemangiomas or hemangiosarcomas at all sites) in male mice. The models were run using the default restrictions on parameters built into the BMD software. Goodness-of-fit was evaluated using the chi-square statistic calculated by the BMDS program. Local fit was evaluated visually on the graphic output, by comparing the observed and estimated results at each data point.

Table A-5. Incidence of Vascular Tumors in Male B6C3F$_1$ Mice Exposed to 4-Nitroaniline by Gavage for 2 Years[a]

Lesion Type	Adjusted Daily Exposure (mg/kg-day)[b]			
	0	2.1	21.4	71.4
Hemangiosarcoma (liver)[c]	0/50[e,f]	1/50 (2%)	2/50 (4%)	4/50[g] (8%)
Hemangiosarcoma or Hemangioma (all sites)[d]	5/50[f] (10%)	3/50 (6%)	4/50 (8%)	10/50[g] (20%)

[a]NTP, 1993
[b]Actual treatment was administered on 5 of 7 days
[c]Historical control data for hemangiosarcoma (liver) in male mice: incidence = 15/699; mean = 2.1%; range = 0−6%
[d]Historical control data for hemangiosarcoma or hemangioma (all sites) in male mice: incidence = 46/700; mean = 6.6%; range = 0−12%
[e]Number of mice examined/number of mice with lesions
[f]Statistically significant positive trend
[g]Statistically significant in pairwise test versus historical control

Modeling results are shown in Table A-6 and Figure A-6 & A-7. Adequate model fit was obtained for the hemangiosarcoma (liver) and hemangiomas or hemangiosarcomas (all sites) incidence data using the multistage model. Model results for hemangiosarcomas (liver) yielded a BMD_{10} of 89 mg/kg-day and a $BMDL_{10}$ of 44 mg/kg-day. Model results for hemangiomas or hemangiosarcomas (all sites) yielded a BMD_{10} of 62 mg/kg-day and a $BMDL_{10}$ of 31 mg/kg-day (see Table A-6). The BMD model results for hemangiomas or hemangiosarcomas (all sites) yielded a $BMDL_{10}$ of 31 mg/kg-day, a value that is lower than the $BMDL_{10}$ for hemangiosarcomas (liver). Based on these considerations the lower $BMDL_{10}$ of 31 mg/kg for the hemangiomas or hemangiosarcomas (all sites) is selected for derivation of the final OSF because it represents a more sensitive indicator of tumorigenicity.

Table A-6. Goodness of Fit Statistics and BMD_{10} and $BMDL_{10}$ Values for Dichotomous Models for Hemangiosarcomas (Liver) and Hemangiomas or Hemangiosarcomas (All Sites) in Male Mice Exposed to Oral 4-Nitroaniline for 2 Years[a]

Model	Goodness of fit *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Hemangiosarcomas (liver)				
Multi-Stage 1-Degree[c]	0.6228	59.75	89	44
Hemangiomas and Hemangiosarcomas (All Sites)				
Multi-Stage 1-Degree[c]	0.7309	137.73	62	31

[a]NTP, 1993
[b]Values >0.1 meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0

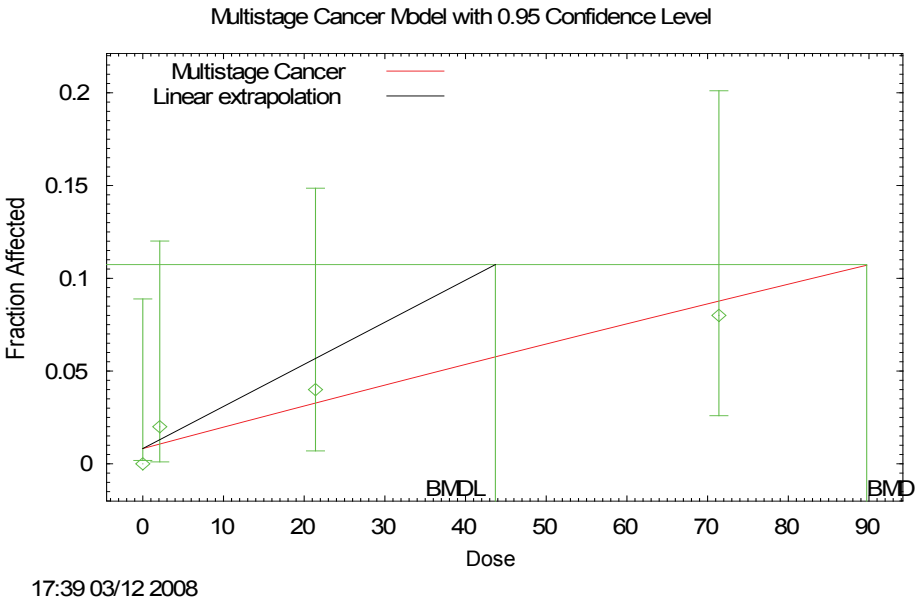

Figure A-6. Observed and Predicted Incidences Of Hemangiosarcomas (Liver) in Male Mice Exposed to Oral 4-Nitroaniline for 2 Years by NTP (1993)

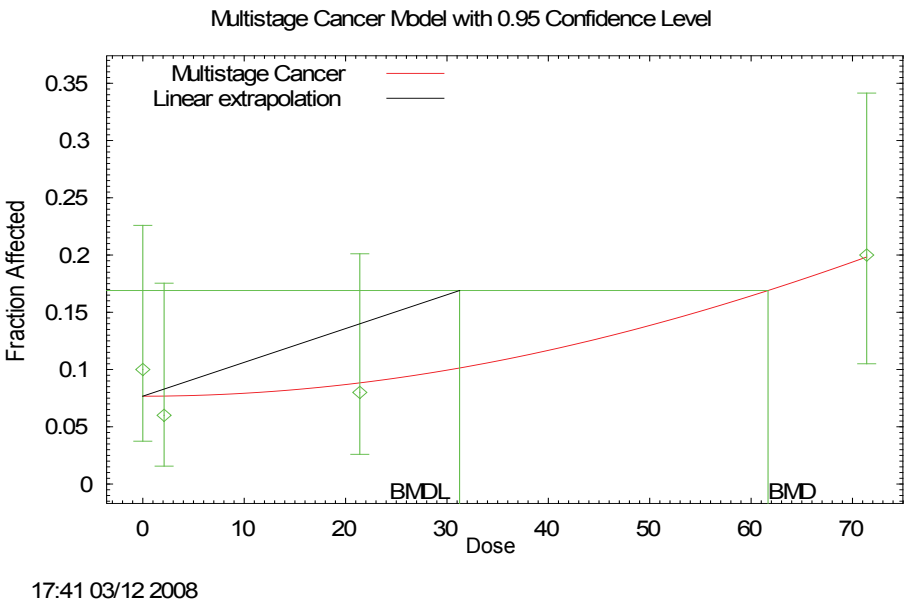

Figure A-7. Observed and Predicted Incidences of Hemangiomas or Hemangiosarcomas (All Sites) in Male Mice Exposed to Oral 4-Nitroaniline for 2 Years by NTP (1993)