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# Provisional Peer-Reviewed Toxicity Values for

# 3-Nitroaniline
(CASRN 99-09-2)

Superfund Health Risk Technical Support Center
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# ACRONYMS AND ABBREVIATIONS

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

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

# PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR 3-NITROANILINE (CASRN 99-09-2)

## Background

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

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

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

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

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

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's 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 U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

## Disclaimers

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

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

## Questions Regarding PPRTVs

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

# INTRODUCTION

3-Nitroaniline is an intermediate in azo dyes (Benya and Cornish, 1994). The empirical formula for 3-nitroaniline is $C_6H_6N_2O_2$ (Figure 1).

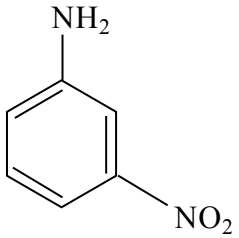


**Figure 1. 3-Nitroaniline Structure**

The U.S. Environmental Protection Agency'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 3-nitroaniline. Subchronic or chronic RfDs or RfCs for 3-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 Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994) includes a Health and Environmental Effects Profile (HEEP) for nitroanilines (U.S. EPA, 1985) and a Health and Environmental Effects Document (HEED) for 3-nitroaniline (U.S. EPA, 1991b) that contain no data regarding oral or inhalation toxicity or carcinogenicity of 3-nitroaniline. No standards for occupational exposure to 3-nitroaniline have been established by the American Conference of Governmental Industrial Hygienists (ACGIH, 2006), the National Institute for Occupational Safety and Health (NIOSH, 2006), or the Occupational Safety and Health Administration (OSHA, 2006). The Agency for Toxic Substances and Disease Registry (ATSDR, 2006), the International Agency for Research on Cancer (IARC, 2006) and the World Health Organization (WHO, 2006) have not published toxicological reviews on nitroanilines or 3-nitroaniline. Toxicity reviews on aromatic nitro, amino and nitro-amino compounds (Weisburger and Hudson, 2001; Woo and Lai, 2001) were consulted for relevant information.

Literature searches for studies relevant to the derivation of provisional toxicity values for 3-nitroaniline (CASRN 99-09-2) 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).

## REVIEW OF PERTINENT DATA

### Human Studies

No studies investigating the effects of subchronic or chronic oral or inhalation exposure to 3-nitroaniline in humans were identified.

### Animal Studies

#### *Oral Exposure*

Studies evaluating the subchronic, chronic, developmental or reproductive toxicity of oral 3-nitroaniline were not located in the published literature. As summarized below, two unpublished toxicity studies, a 28-day repeated dose study and a short-term reproductive/developmental toxicity study, conducted by the Japanese Ministry of Health and Welfare were identified in the Screening Information Data Set on 3-nitroaniline prepared by the Organization for Economic Cooperation Development (OECD/SIDS, 1994). Translations of these studies were made available by EPA.

**Short-term Study—**A 28-day repeated dose study was conducted by the Japanese Ministry of Health and Welfare; information provided in this summary was obtained from Tables 1−7 of the translated report (Onodera, ND) and from the OECD/SIDS (1994) summary. Groups of 5 male and 5 female Crj:F344 rats were administered 0, 15, 50 or 170 mg/kg 3-nitroaniline (99.8% pure) daily by gavage in olive oil for 28 days. In the control and 170 mg/kg-day groups, additional rats (5/sex/group) were dosed for 28 days followed by a 14-day recovery period. The study followed the Japanese Guideline for 28 Day Repeated Dose Toxicity Test of Chemicals, but was not conducted using GLP. Animals were observed daily for mortality and clinical signs. Body weights were recorded daily and food consumption was recorded weekly. At the end of the treatment and recovery periods, blood samples were collected and analyzed for hematology [red blood cell (RBC) count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell (WBC) count with differential, reticulocyte count and methemoglobin (MetHgb)] and clinical chemistry [activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), γ-glutamyl aminotransferase (GGT), and choline esterase; levels of total cholesterol, total protein, albumin, blood urea nitrogen (BUN), creatinine and electrolytes, as well as albumin/globulin (A/G) ratio]; urine was collected and analyzed for pH, protein, bilirubin, blood, ketone bodies and glucose. Organ weights were recorded and necropsy and comprehensive histopathological examinations were performed on all animals at the end of the treatment and recovery periods.

No mortalities occurred during the treatment or recovery periods. The male rats receiving the high dose seem to have significantly lower body weight compared to the control, but no such changes were seen in males in other dose groups or females in all dose group. There was limited information regarding food and water consumption in the available report. Cyanosis was observed in male and female rats in the 170 mg/kg-day group (incidence data not reported), but not in the 15 or 50 mg/kg-day groups. At the end of the 28-day treatment period, body weight was significantly decreased by 9.5% compared to controls in males treated with 170 mg/kg-day, but not in males in the 15 or 50 mg/kg-day groups or females in any treatment group; at the end of the 14-day recovery period, body weight in males in the 170 mg/kg-day group remained decreased (6.4%, $p \leq 0.01$). Treatment-related effects on hematology parameters findings were consistent with the effects of increased blood concentrations of methemoglobin (a form of hemoglobin that does not bind oxygen); specifically, accelerated red blood cell destruction (hemolytic anemia) and compensatory erythropoiesis to maintain erythrocyte mass (Table 1). At the end of the treatment period, MetHgb was detected in male rats in the 170 mg/kg-day group, but not in the control, 15 or 50 mg/kg-day groups. In male rats, dose-dependent decreases were observed for RBC count, blood Hgb and Hct in all 3-nitroaniline treatment groups; at doses of ≤50 mg/kg-day, MCHC was decreased and reticulocytes were increased; in the 170 mg/kg-day group, MCHC was decreased and MCV, MCH, erythroblasts and WBC count were increased. At the end of the recovery period, all hemotology parameters in males in the 170 mg/kg-day group returned to control levels, except for increased MCV (18.2% increase, $p \leq 0.05$) and MCH (18.8% increase, $p \leq 0.05$).

In female rats, MetHgb was detected in the 50 and 170 mg/kg-day groups, but not in the control or 15 mg/kg-day groups. Dose-dependent decreases in RBC count, blood hemoglobin and Hct were observed in females in all 3-nitroaniline groups; MCV, MCH and reticulocytes were increased at doses ≥50 mg/kg-day; MCHC was decreased and erythrocytes, WBC count and platelets were increased in the 170 mg/kg-day group. At the end of the recovery period, the following hematology parameters in female rats treated with 170 mg/kg-day were significantly increased compared to control levels: Hgb (11.6%), Hct (12.7%), MCV (16.4%), MCH (15.6%) and WBC count (35.4%). Small changes in clinical chemistry parameters were generally consistent with mild hemoconcentration (Table 1). In male rats, effects included increased levels of cholesterol (≥50 mg/kg-day), protein (≥50 mg/kg-day), and albumin (≥50 mg/kg-day), as well as A/G ratio (170 mg/kg-day). In females, effects included increased levels of cholesterol (15 and 50 mg/kg-day), protein (≥15 mg/kg-day), albumin (≥15 mg/kg-day), and BUN (≥15 mg/kg-day), as well as A/G ratio (170 mg/kg-day).

Changes in absolute and relative organ weights in male and female rats treated with oral 3-nitroaniline are summarized in Table 2. In male rats, relative spleen and liver weights were significantly increased in all 3-nitroaniline groups and absolute spleen and liver weights were significantly increased at doses ≥50 mg/kg-day. Relative kidney weights were slightly increased in the 170 mg/kg-day group. Absolute and relative testes weights were significantly decreased in males treated with 170 mg/kg-day, but not lower doses. Although absolute and relative thyroid weights were slightly increased in the 15 and 170 mg/kg-day groups, changes in thyroid weight were not significantly different from controls in the 50 mg/kg-day group. Following the 14-day recovery period, absolute and relative spleen weights remained increased and testes weight remained decreased compared to control. Although no treatment-related effects on absolute and relative epididymis weights were observed at the end of the treatment period, absolute and relative epididymis weights were significantly decreased by 31.9% and 28.6%, respectively, compared to controls after the 14-day recovery period in males dosed with 170 mg/kg-day. In female rats, increases in absolute and relative spleen weights (≥50 mg/kg-day) and absolute and relative liver weights (≥15 mg/kg-day) were observed at the end of the treatment period. Absolute and relative kidney weights were slightly, but significantly, increased in females treated with 170 mg/kg-day. At the end of the recovery period, absolute and relative spleen and liver weight remained elevated compared to controls in females in the 170 mg/kg-day group. Absolute and relative right ovary weights were slightly increased by 21.4% ($p < 0.05$) and 23.5% ($p < 0.01$) at the end of the recovery period, although no treatment-related changes were observed at the end of the treatment period.

**Table 1. Selected Hematology and Clinical Chemistry Parameters in Crj:F344 Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Parameter | Exposure Group (mg/kg-day) | | | |
|---|---|---|---|---|
| | 0 | 15 | 50 | 170 |
| **Males** | | | | |
| MetHgb (%) | 0 | 0 | 0 | 3.26 |
| RBC ($10^4$/µL) | 934 ± 39.2[b] | 868 ± 12.0[d] | 781 ± 16.8[d] | 494 ± 27.0[d] |
| Hgb (g/dL) | 15.6 ± 0.23 | 14.5 ± 0.21[d] | 14.4 ± 0.31[d] | 11.8 ± 0.52[d] |
| Hct (%) | 52.5 ± 2.5 | 48.9 ± 0.8[d] | 48.2 ± 1.1[d] | 42.7 ± 1.7[d] |
| MCV (fL) | 56.1 ± 0.6 | 56.4 ± 0.3 | 61.7 ± 0.5 | 86.7 ± 1.5[c] |
| MCH (ρg) | 16.7 ± 0.5 | 16.7 ± 0.1 | 18.5 ± 0.4 | 24.0 ± 0.5[c] |
| MCHC (g/dL) | 29.8 ± 1.0 | 29.5 ± 0.2 | 29.9 ± 0.5 | 27.7 ± 0.3[c] |
| Reticulocytes (%RBC) | 3.48 ± 0.38 | 3.54 ± 0.86 | 5.50 ± 0.67[d] | 39.7 ± 1.29[d] |
| WBC ($10^2$/µL) | 44.8 ± 4.4 | 43.6 ± 4.8 | 49.0 ± 5.1 | 354 ± 72.7[c] |
| Erythroblasts (%WBC) | 0.8 ± 1.5 | 3.0 ± 3.6 | 4.2 ± 4.7 | 18.5 ± 9.8[c] |
| Cholesterol (mg/dL) | 51.6 ± 6.3 | 60.2 ± 2.7 | 75.0 ± 9.0[d] | 79.8 ± 10.1[d] |
| Protein (g/dL) | 5.88 ± 0.29 | 6.08 ± 0.13 | 6.30 ± 0.10[d] | 6.52 ± 0.23[d] |
| Albumin (g/dL) | 4.64 ± 0.18 | 4.82 ± 0.16 | 5.08 ± 0.08[d] | 5.38 ± 0.26[d] |
| A/G ratio | 3.78 ± 0.44 | 3.84 ± 0.39 | 4.20 ± 0.46 | 4.84 ± 0.96[c] |
| **Females** | | | | |
| MetHgb (%) | 0 | 0 | 0.04 | 3.16 |
| RBC ($10^4$/µL) | 930 ± 47.2 | 858 ± 31.3[d] | 769 ± 18.5[d] | 480 ± 15.2[d] |
| Hgb (g/dL) | 15.8 ± 0.67 | 14.7 ± 0.40[d] | 14.4 ± 0.31[d] | 11.1 ± 0.37[d] |
| Hct (%) | 51.3 ± 2.6 | 48.0 ± 1.4[c] | 46.4 ± 0.7[d] | 40.5 ± 1.0[d] |
| MCV (fL) | 55.2 ± 0.5 | 56.0 ± 0.9 | 60.4 ± 0.7[d] | 84.4 ± 1.4[d] |
| MCH (ρg) | 17.1 ± 0.3 | 17.2 ± 0.2 | 18.7 ± 0.3[d] | 23.0 ± 0.1[d] |
| MCHC (g/dL) | 30.9 ± 0.6 | 30.7 ± 0.1 | 31.0 ± 0.3 | 27.3 ± 0.5[d] |
| Reticulocytes (%RBC) | 1.78 ± 0.37 | 3.72 ± 0.29 | 5.92 ± 0.40[c] | 41.3 ± 3.86[c] |
| WBC ($10^2$/µL) | 40.2 ± 11.9 | 37.8 ± 4.8 | 40.8 ± 8.0 | 320 ± 41.2[c] |
| Erythroblasts (%WBC) | 1.3 ± 1.5 | 1.5 ± 3.3 | 3.9 ± 3.4 | 13.5 ± 4.3[d] |
| Platelet count ($10^4$/µL) | 94.9 ± 11.9 | 101 ± 5.3 | 97.4 ± 4.2 | 81.6 ± 4.9[d] |

**Table 1. Selected Hematology and Clinical Chemistry Parameters in Crj:F344 Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Parameter | Exposure Group (mg/kg-day) | | | |
|---|---|---|---|---|
| | **0** | **15** | **50** | **170** |
| Cholesterol (mg/dL) | 70.8 ± 8.3 | 89.4 ± 4.9[d] | 84.0 ± 3.2[d] | 76.2 ± 6.2 |
| Protein (g/dL) | 5.70 ± 0.21 | 6.32 ± 0.18[d] | 6.28 ± 0.11[d] | 6.32 ± 0.23 [d] |
| Albumin (g/dL) | 4.50 ± 0.21 | 5.04 ± 0.23 [d] | 5.10 ± 0.07[d] | 5.28 ± 0.16[d] |
| A/G ratio | 3.76 ± 0.17 | 3.96 ± 0.42 | 4.36 ± 0.47 | 5.12 ± 0.55[d] |
| BUN (mg/dL) | 10.2 ± 0.88 | 12.8 ± 1.35[d] | 12.4 ± 0.88 [d] | 13.5 ± 0.74[d] |

[a]Onondera, ND
[b]Means ± SD
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

**Table 2. Selected Absolute and Relative Organ Weights in Crj:F344 Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Parameter | Exposure Group (mg/kg-day) | | | |
|---|---|---|---|---|
| | 0 | 15 | 50 | 170 |
| **Males** | | | | |
| Absolute spleen weight (g) | 0.48 ± 0.06[b] | 0.56 ± 0.03 | 0.67 ± 0.03[d] | 1.77 ± 0.10[d] |
| Relative spleen weight (%) | 0.23 | 0.26[d] | 0.33[d] | 0.94[d] |
| Absolute liver weight (g) | 6.17 ± 0.16 | 6.74 ± 0.25[d] | 7.28 ± 0.24[d] | 8.14 ± 0.27[d] |
| Relative liver weight (%) | 2.94 | 3.18[d] | 3.60[d] | 4.3[d] |
| Relative right kidney weight (%) | 0.34 | 0.38 | 0.36 | 0.39[c] |
| Relative left kidney weight (%) | 0.36 | 0.36 | 0.37 | 0.40[d] |
| Absolute right testes weight (g) | 1.33 ± 0.06 | 1.39 ± 0.05 | 1.21 ± 0.17 | 0.61 ± 0.09[d] |
| Relative right testes weight (%) | 0.64 | 0.66 | 0.60 | 0.32[d] |
| Absolute left testes weight (g) | 1.33 ± 0.05 | 1.40 ± 0.02 | 1.28 ± 0.21 | 0.60 ± 0.09[e] |
| Relative left testes weight (%) | 0.64 | 0.66 | 0.63 | 0.32[e] |
| Absolute thyroid weight (g) | 13 ± 8.4 | 16 ± 2.2[c] | 13 ± 1.2 | 16 ± 2.1[c] |
| Relative thyroid weight (%) | 0.006 | 0.008[c] | 0.006 | 0.009[d] |
| **Females** | | | | |
| Absolute spleen weight (g) | 0.36 ± 0.02 | 0.42 ± 0.02 | 0.54 ± 0.03[c] | 1.63 ± 0.12[c] |
| Relative spleen weight (%) | 0.25 | 0.27 | 0.37[c] | 1.12[c] |
| Absolute liver weight (g) | 3.94 ± 0.33 | 4.61 ± 0.14[d] | 5.27 ± 0.18[d] | 6.77 ± 0.20[d] |
| Relative liver weight (%) | 2.66 | 3.00 | 3.60[c] | 4.63[c] |
| Absolute right kidney weight (%) | 0.53 ± 0.04 | 0.55 ± 0.03 | 0.54 ± 0.03 | 0.60 ± 0.02[d] |
| Relative right kidney weight (%) | 0.35 | 0.36 | 0.37 | 0.41[d] |
| Absolute left kidney weight (%) | 0.53 ± 0.04 | 0.57 ± 0.04 | 0.54 ± 0.01 | 0.60 ± 0.04[c] |
| Relative left kidney weight (%) | 0.35 | 0.37 | 0.37 | 0.41[c] |

[a]Onondera, ND
[b]Means or means ± SD
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)
[e]Not marked as statistically significant in the original report, but similarity to data for right testes suggests this might be an error in the report

Histopathological findings in spleen, bone marrow and liver were consistent with hemolytic anemia and compensatory hematopoiesis; however, incidence data were not reported for any histopathological findings and results were not reported separately for males and females. In the spleen, hemosiderin deposition, extramedullary hematopoiesis and congestion were observed in all 3-nitroanline groups; lesion severity increased with dose from slight at 15 mg/kg-day to severe at 170 mg/kg-day. Erythroid hyperplasia of bone marrow was observed in all treatment groups, with dose-related severity as in the spleen. Hepatocyte swelling was observed in rats treated with ≥50 mg/kg-day and hepatic hemosiderin deposition and extramedullary hematopoiesis were observed in rats treated with 170 mg/kg-day. Renal lipofuscin (a brownish, fat-soluble pigment) deposition (mainly in the proximal renal tubules) was observed in the 50 and 170 mg/kg-day groups. Effects on male reproductive organs were observed primarily in the 170 mg/kg-day group, including a reduction in spermatogenesis with multinucleated giant cell formation in the testes and the absence of spermatozoa in the epididymis. With the exception of hemosiderin deposition of the spleen and liver, the severity of all lesions was decreased after the 14-day recovery period in the 170 mg/kg-day group. Based on hematological effects (decreased RBC count and hemoglobin) and histopathological finding of spleen (hemosiderin deposition, extramedullary hematopoiesis and congestion) and bone marrow (erythroid hyperplasia) observed in all 3-nitroaniline treatment groups, a LOAEL of 15 mg/kg-day was identified; a NOAEL was not established.

**Reproduction/Developmental Study—**A short-term reproductive/developmental toxicity study was conducted by the Japanese Ministry of Health and Welfare; information provided in this summary was obtained primarily from Tables 1−9 of the translated report (Mizutani, ND) and from the OECD/SIDS (1994) summary. Groups of 13 male and 13 female Crj:CD(SD) rats were administered 0, 5, 15 or 50 mg/kg of 3-nitroaniline (99% pure) in CMC[1]-Na aqueous solution by daily gavage from 14 days before mating, through a 14-day mating period, and a 14-day post-mating period (males) or through day 4 of lactation (females). The study followed the OECD Preliminary Reproductive/Developmental Toxicity Screen Test protocol and was conducted under GLP conditions. Animals were observed daily for mortality and clinical signs. Adult body weights and food consumption were recorded weekly. Mating performance and fertility parameters (copulation rate, time to copulation, number of fertile copulations, times of vaginal estrous, number of fertile females and number of fertile copulations) and reproductive parameters (duration of gestation, number of *corpora lutea*, implantations and resorptions, litter size, sex distribution, live birth index and pup survival) were evaluated. Upon completion of treatment, complete gross pathological examination and histopathological examination of the ovaries, testes and epididymides of parental animals were performed; hematology, clinical chemistry and organ weights were not evaluated. Body weights of pups were recorded at birth and on post-natal day (PND) 4 and pup survival from birth to PND 4 was evaluated; on PND 4, pups were examined for external, internal and skeletal malformations.

[1] The definition of CMC-Na solution was not found in the available translated report (Mizutani, ND) or OECD/SIDS (1994). Presumably, CMC-Na solution stands for sodium carboxymethyl cellulose solution.

During delivery on the 23rd day of gestation, 1 female in the 50 mg/kg-day group died; no treatment-related clinical signs of toxicity were observed prior to death. A female in the 15 mg/kg-day group and 2 females in the 50 mg/kg-day group showed signs of difficult labor and lost their entire litters. In the 50 mg/kg-day group, one female exhibited pale extremities "late" in the dosing period. Mortality was not observed in males in any treatment group; one male in the 50 mg/kg-day group exhibited pale extremities "late" in the dosing period. Body weights of adult males and females in the 50 mg/kg-day group were slightly, but consistently, decreased compared to controls during the treatment period; however, decreases did not reach statistical significance. Mating performance and fertility were unaffected by treatment at any dose. The live birth index was decreased in the 15 mg/kg-day (87.9%) and 50 mg/kg-day (79.8% viability) groups, compared to controls (95.8%); however, offspring loss appeared related to litter loss resulting from difficult labors, as discussed above. All other reproductive parameters were comparable to controls for all 3-nitroaniline treatment groups. There were no treatment-related effects on pup survival, body weights at birth or on PND 4 or morphological development.

Gross pathological examination revealed effects to the liver and spleen of adult males and to the spleen of adult females. On necropsy, 3 males in the 15 mg/kg-day group and all males in the 50 mg/kg-day group had enlarged and/or dark-colored spleen; no gross pathological findings were observed in controls or males treated with 5 mg/kg-day. Hepatomegaly was observed in 3 males in the 50 mg/kg-day group, but not in the control, 5 or 15 mg/kg-day groups. In females, enlarged and dark-colored spleens were observed in 1 rat in the 15 mg/kg-day group and 8 rats in the 50 mg/kg-day group, compared to none in the control or 5 mg/kg-day groups. No treatment-related histopathological changes in the ovaries, testes or epididymides of parental animals were observed. Based on gross pathological findings of the spleen (dark red color) in male rats, and potential reproductive toxicity (signs of difficult labor and loss of litters) in female rats, NOAEL and LOAEL values of 5 and 15 mg/kg-day, respectively, for toxicity to the parental generation were identified. However, the absence of evaluation of hematological parameters dictates caution in interpreting the parental NOAEL, since a NOAEL for hematological effects was not established in the 28-day repeated dose study. For fetal effects, a NOAEL of 50 mg/kg-day was identified; a LOAEL was not established.

### *Inhalation Exposure*

No subchronic, chronic, developmental or reproduction studies on inhaled 3-nitroaniline in animals were identified**.**

## Other Studies

### *Studies Comparing 3- and 4-Nitroaniline*

Methemoglobinemia has been identified as a primary adverse effect of subchronic and chronic oral exposure to other aniline and substituted aniline compounds, including 4-nitroaniline (NTP, 1993). 3- and 4-Nitroaniline appear to be nearly equivalent in their potency to convert hemoglobin to methemoglobin, based on results of *in vitro* and acute *in vivo* studies. Watanabe et al. (1976) measured the percent conversion of hemoglobin to methemoglobin in

male Wistar rats 5 hours after treatment with a 100 μmole/kg i.p. dose of 3- or 4-nitroaniline. *In vitro* studies were also performed, in which 0.1 μmole of hemoglobin was incubated with 0.5 μmole of 3- or 4-nitroaniline for 5 hours. The extent of conversion appeared to be similar for both isomers; 3-nitroaniline converted 12.9 and 5.1% of the hemoglobin *in vivo* and *in vitro* and 4-nitroaniline converted 11.0 and 5.7% *in vivo* and *in vitro*, respectively. SOCMA (1984) gave single oral 150 or 600 mg/kg doses of 3- or 4-nitroaniline in corn oil to male Sprague-Dawley rats and measured the percent of conversion of hemoglobin to methemoglobin at 1 and 6 hours after treatment. Similar levels of methemoglobin were formed for each isomer (Table 3). In another *in vitro* study, French et al. (1995) compared methemoglobin formation in freshly-drawn sheep erythrocytes treated for 1 hour with 3- or 4-nitroaniline, with or without the presence of an NADP-bioactivation system (glucose, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and S9). Without activation, both isomers increased the level of methemoglobin formation to a similar degree; methemoglobin concentrations were 1.6% in untreated control blood and 3.6% or 3.4% in blood treated with 1 mM concentrations of 3-nitroaniline or 4-nitroaniline, respectively. At the same concentrations with activation, the amount of methemoglobin (unchanged in control blood) was significantly increased by treatment with either isomer, with 4-nitroaniline having about twice the activity of 3-nitroaniline: 25.3% and 9.7% methemoglobin, respectively.

**Table 3. Comparison of Methemoglobin Formation in Sprague-Dawley Rats Exposed to Single Doses of 3-Nitroaniline and 4-Nitroaniline**[a]

| Compound | Dose (mg/kg) | Percent Hemoglobin Conversion | |
|---|---|---|---|
| | | 1 Hour | 6 Hours |
| 3-Nitroaniline | 150 | 23.5 | 10.5 |
| | 600 | 35.6 | 28.9 |
| 4-Nitroaniline | 150 | 20.1 | 11.6 |
| | 600 | 40.8 | 32.0 |

[a]SOCMA, 1984

Oral $LD_{50}$ data suggest that 3-nitroaniline may be somewhat more toxic than the 4-isomer on an acute basis. Vernot et al. (1977) estimated oral $LD_{50}$ values for 3-nitroaniline of 540 mg/kg in male rats and 310 mg/kg in mice; corresponding values for 4-nitroaniline were 3250 and 810 mg/kg. Moskalenko (1966) reported oral $LD_{50}$ values for 3-nitroaniline of 450 and 700 mg/kg for guinea pigs and mice, respectively; corresponding values for 4-nitroaniline were 450 and 1500 mg/kg. The agonal signs associated with the two isomers differed slightly; 4-nitroaniline produced spasms and 3-nitroaniline produced "inhibition" (not otherwise described). Eastman Kodak Co. (1969) reported oral $LD_{50}$ values in rats of 50−400 mg/kg for 3-nitroaniline and 400−3200 mg/kg for 4-nitroaniline. Vasilenko et al. (1974a,b) reported oral $LD_{50}$ values in rats of 900 and 1410 mg/kg for 3- and 4-nitroaniline, respectively. When given at an oral dose of 50% of the $LD_{50}$, the 4-isomer was more potent than the 3-isomer in inducing methemoglobinemia and sulfhemoglobinemia (Vasilenko et al., 1974a).

**Genotoxicity Studies**

The genotoxicity attributed to 3-nitroaniline has been investigated in bacterial systems in several studies. Results of reverse mutation assays in *Salmonella typhimurium* show that with metabolic activation, 3-nitroaniline induced mutations (Dellarco and Prival, 1989; Kawai et al., 1987; Shimizu and Yano, 1986; Shahin, 1985; Thompson et al., 1983; Chiu et al., 1978; Garner and Nutman, 1977), although one study in *Escherichia coli* reported negative results with metabolic activation (data without activation not reported) (Thompson et al., 1983). Studies in *S. typhimurium* conducted without metabolic activation have yielded both negative (Abmann et al., 1997; Shahin, 1985; Chiu et al., 1978; Garner and Nutman, 1977) and positive (Abmann et al., 1997; Kawai et al., 1987; OECD/SIDS, 1994; Sofuni, NDa; Shimizu and Yano, 1986; Shahin, 1985; Chiu et al., 1978) results, depending upon the *S. typhimurium* strain tested. 3-Nitroaniline was weakly mutagenic in the Kada *Bacillus subtilis* rec assay without activation (Shimizu and Yano, 1986). These results suggest that the mutagenicity attributed to 3-nitroaniline is increased with metabolic activation.

Few data on the genotoxicity of 3-nitroaniline in mammalian cells are available. 3-Nitroaniline tested negative for unscheduled DNA synthesis in cultured rat hepatocytes (Thompson et al., 1983). The Japanese Ministry of Health and Welfare conducted an *in vitro* chromosome aberration test in Chinese hamster CHL cells (Sofuni, NDb) and an *in vivo* micronucleus test in mice (Shibuya, ND); information was provided in a translation of study summaries and from OECD/SIDS (1994). Positive results were observed in the chromosome aberration test in the absence and presence of metabolic activation. 3-Nitroaniline tested positive in the micronucleus test when mice (Crj:BDF1 strain) were treated with a single oral dose of 300 mg/kg; inhibition of bone marrow cell proliferation was not observed under test conditions. Negative results were observed following oral administration of 75 or 150 mg/kg.

## FEASIBILITY OF DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR 3-NITROANILINE

Studies evaluating subchronic or chronic oral exposure to 3-nitroaniline in humans were not identified from the published literature. There were two unpublished studies, a 28-day toxicity study and a 42-day reproductive/developmental toxicity screening study, that were conducted by the Japanese Ministry of Health and Welfare; data were obtained from tables provided in translations of original study reports (Onodera, ND; Mizutani, ND) and from the OECD/SIDS (1994) summaries. These studies appear to have been adequately conducted.

Results of the 28-day toxicity study, which evaluated comprehensive endpoints, identified the blood as the primary target organ for oral exposure to 3-nitroaniline. Observed effects in the blood were consistent with methemoglobinemia, anemia and compensatory hematopoiesis. Histological observations indicative of anemia and compensatory hematopoiesis included findings in the spleen (hemosiderin deposition, extramedullary hematopoiesis, and congestion) and bone marrow (erythroid hyperplasia) at doses ≥15 mg/kg-day and hepatic extramedullary hematopoiesis at a dose of 170 mg/kg-day. Increased absolute and relative liver

and spleen weights were observed at doses ≥15 mg/kg-day and may have been secondary responses to hemolytic anemia and compensatory erythropoiesis. 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. Due to the presence of precipitated hemoglobin, erythrocytes are prematurely removed from blood by the spleen, resulting in hemolytic anemia. As a compensatory response to methemoglobin-induced functional and hemolytic anemia, hematopoiesis is increased. Methemoglobinemia, hemolytic anemia and compensatory erythropoiesis also have been identified as the primary adverse effects of subchronic and chronic oral exposure to other aniline and substituted aniline compounds, including 4-nitroaniline (NTP, 1993).

Other effects observed in rats exposed to 3-nitroaniline for 28 days included effects on male reproductive organs and changes in clinical chemistry parameters. Effects on male reproductive organs were observed in the 170 mg/kg-day group and included testicular atrophy, reduction in spermatogenesis with multinucleated giant cell formation in the testes and the absence of spermatozoa in the epididymis. However, since hematological effects occurred at lower doses (≥15 mg/kg-day), effects on male reproductive organs were not considered as the basis of the subchronic and chronic p-RfD. Minor changes in clinical chemistry parameters (increased levels of cholesterol, protein, albumin and BUN) observed in female rats administered ≥15 mg/kg-day and male rats administered ≥50 mg/kg-day were consistent with mild hemoconcentration, rather than a specific toxic effect.

The 42-day reproductive/developmental screening study reported no evidence for fetal effects (body weight, survival or malformations) at 3-nitroaniline doses up to 50 mg/kg-day, yielding a NOAEL for fetal toxicity of 50 mg/kg-day (a LOAEL was not identified). In parental animals, NOAEL and LOAEL values of 5 and 15 mg/kg-day, respectively, were identified based on gross pathological findings of the spleen (dark red color) in male rats, and potential reproductive toxicity (signs of difficult labor and loss of litters) in female rats; however, comprehensive endpoints (hematology, clinical chemistry, comprehensive histopathological examination) were not examined, dictating caution in interpretation of the parental NOAEL.

Based on the available data, anemia was identified as the most sensitive effect following oral exposure to 3-nitroaniline and, therefore, selected as the basis of the subchronic and chronic p-RfDs. The most sensitive measures of hematological effects were RBC count, blood hemoglobin concentration and hematocrit (≥15 mg/kg-day). Dose-response modeling was performed for RBC count and hemoglobin. Hematocrit was not modeled because it is a less direct measure of effect (typically calculated rather than measured). Histopathological changes of the spleen and bone marrow secondary to anemia and compensatory hematopoiesis were observed at the same doses as hematological effects. However, due to the absence of incidence data, it was not possible to perform dose-response modeling for the histological data.

To determine the point of departure (POD) for derivation of the subchronic and chronic p-RfDs, data sets for RBC counts and hemoglobin concentration in male and female rats (Table 4) were first evaluated for suitability for benchmark dose (BMD) modeling using the EPA Benchmark Dose Software (BMDS) version 1.4.1 (U.S. EPA, 2007). Continuous-variable models in the EPA BMDS (version 1.4.1) were fit to the data using a default benchmark response of 1 SD above the control mean to estimate the benchmark dose, as recommended by U.S. EPA (2000). If data were considered suitable for modeling by benchmark dose analysis, the POD would be identified as the lowest BMDL (e.g., lower confidence limit (95%) on the benchmark dose) for the best fitting model. If data were not suitable for benchmark dose analysis, the POD would be based on a NOAEL/LOAEL approach. Details of the BMD analysis are presented in Appendix B.

Adequate BMD model fit was achieved for only the female RBC count data (Appendix B). A $BMD_{1SD}$ of 10.8 mg/kg-day and $BMDL_{1SD}$ of 7.5 mg/kg-day were determined from the female rat data. Models available in the BMDS could not adequately fit the male RBC count or blood hemoglobin concentration data, or the female blood hemoglobin data, even after dropping the high-dose group; therefore, the potential POD based on these endpoints would be LOAEL of 15 mg/kg-day. Comparing to the estimated BMDL of 7.5 mg/kg-day for decreased RBC in female rats, the more conservative POD from these endpoints (decreases in RBC and hemoglobin in male and female rats) would be the LOAEL of 15 mg/kg-day considering an application of an extra uncertainty factor of 10 for extrapolation from LOAEL to NOAEL.

**Table 4. Red Blood Cell Counts and Blood Hemoglobin Concentrations in Crj:F344 Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Parameter | Exposure Group (mg/kg-day) | | | |
|---|---|---|---|---|
| | 0 | 15 | 50 | 170 |
| **Males** | | | | |
| RBC ($10^4$/μL) | 934 ± 39.2[b] | 868 ± 12.0[c] | 781 ± 16.8[c] | 494 ± 27.0[c] |
| Hgb (g/dL) | 15.6 ± 0.23 | 14.5 ± 0.21[c] | 14.4 ± 0.31[c] | 11.8 ± 0.52[c] |
| **Females** | | | | |
| RBC ($10^4$/μL) | 930 ± 47.2 | 858 ± 31.3[c] | 769 ± 18.5[c] | 480 ± 15.2[c] |
| Hgb (g/dL) | 15.8 ± 0.67 | 14.7 ± 0.40[c] | 14.4 ± 0.31[c] | 11.1 ± 0.37[c] |

[a]Onodera, ND
[b]Means ± SD
[c]Significantly different from control ($p \leq 0.01$)

If the LOAEL of 15 mg/kg-day from the 28-day rat study was used as the POD in the derivation of subchronic and chronic p-RfD, the areas of uncertainty will include an extrapolation from animals to humans, inter human variability, use of a short-term study, use of a LOAEL instead of a NOAEL, and database deficiency. Due to the significant uncertainties involved, derivation of a provisional subchronic or chronic RfD was considered inappropriate. Nevertheless, Appendix A of this document contains a Screening Value that may be useful in certain instances. Please see Appendix A for details.

## FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR 3-NITROANILINE

No studies investigating the effects of subchronic or chronic inhalation exposure to 3-nitroaniline in humans or animals were identified. The lack of suitable data precludes derivation of subchronic and chronic p-RfCs for 3-nitroaniline.

## PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 3-NITROANILINE

### Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to 3-nitroaniline in humans were not identified in the available literature. Cancer bioassays for 3-nitroaniline have not been conducted in animals by either oral or inhalation exposure. Genotoxicity data suggest that 3-nitroaniline has some mutagenic potential. Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), inadequate information is available to assess the carcinogenic potential of 3-nitroaniline.

### Quantitative Estimates of Carcinogenic Risk

Derivation of quantitative estimates of cancer risk for 3-nitroaniline is precluded by the lack of suitable data.

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## APPENDIX A. DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL SCREENING RfD FOR 3-NITROANILINE

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

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

The **subchronic screening RfD of 0.001 mg/kg-day or 1E-03 mg/kg-day** for 3-nitroaniline, based on the LOAEL of 15 mg/kg-day for hematological effects (decreased RBC count and hemoglobin), and histopathological finding of spleen and bone marrow (Onodera, ND), was derived as follows:

**Subchronic screening RfD** = LOAEL ÷ UF
= 15 mg/kg ÷ 10,000
= 0.001 mg/kg-day or **1E-03 mg/kg-day**

The uncertainty factor of 10,000 was composed of the following:

- A 10-fold UF for intraspecies differences was 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, hematopoietic disorders, or low levels of MetHb reductase seen in neonates may be more susceptible to oral 3-nitroaniline.
- A full UF of 10 was applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A partial UF of 3 ($10^{0.5}$) was applied for use of a study with less-than-subchronic exposure duration.
- A 10-fold UF was applied for use of a LOAEL as the point departure.
- A partial UF of 3 ($10^{0.5}$) for database insufficiencies was applied. No subchronic or chronic oral toxicity studies were identified. However, an oral reproductive/ developmental screening study was available.

This value is 10-fold lower than the subchronic p-RfD of 0.01 mg/kg-day for 4-nitroaniline. The lower estimated risk value for 3-nitroaniline is due to significant uncertainties in estimating a subchronic risk value, which include the lack of subchronic or chronic studies, use of LOAEL as a POD, and an inadequate database. As summarized before, oral LD50 data suggest that 3-nitroaniline may be somewhat more toxic than the 4-nitroaniline on an acute basis; however, 3- and 4-nitroaniline appear to be nearly equivalent in their potency to convert hemoglobin to methemoglobin, based on results of in vitro and acute in vivo studies. The adverse responses observed in the critical 28-day rat study for 3-nitroaniline are consistent with those observed in a 14-day mouse study for 4-nitroaniline which identified a LOAEL of 7.1 mg/kg-day for methemoglobinemia. However, a direct comparison of subchronic or chronic toxicity between these two chemicals is difficult due to the lack of data from the same animal species and treatment duration for 3-nitroaniline. As the result, the estimated screening value subchronic RfD for 3-nitroaline is more conservative than the corresponding p-RfD for 4-nitroaniline.

Confidence in the key study is low. Comprehensive endpoints were examined, although a small number of animals per dose-group were examined (5/sex/goup) and the study duration was less than subchronic. The study includes observations of endpoints at multiple dose levels; however, the study did not identify a NOAEL, and the most data were not amenable to BMD modeling. Confidence in the database is low, since no subchronic or chronic oral toxicity studies were identified, although an oral short-term study and a reproductive/ developmental screening study were available (showing no reproductive or developmental effects). The confidence in the subchronic screening RfD for 3-nitroaniline is low.

## APPENDIX B. DETAILS OF BMD ANALYSIS FOR 3-NITROANILINE

The Benchmark Dose model fitting procedure for continuous data is as follows. The BMD modeling was conducted with the EPA's BMD software (BMDS version 1.4.1). For all the continuous data (RBC and hemoglobin), the original data were modeled with all the continuous models available within the software. An adequate fit was judged based on the goodness of fit *p*-value ($p > 0.1$), scaled residual at the range of benchmark response (BMR), and visual inspection of the model fit. 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 determined final use of the model results. If a homogenous variance model was recommended based on statistics (test 2) provided from the BMD model runs, the final BMD results would be estimated from a homogenous variance model. If the test for homogenous variance (test 2) was negative ($p < 0.1$), the model was run again while applying the power model integrated into the BMDS to account for nonhomogenous variance (known as nonhomogenous model). If the nonhomogenous variance model did not provide an adequate fit to the variance data (test 3 *p*-value less than 0.1), the data set would be considered unsuitable for BMD modeling. Among all the models provided adequate data fit, the lowest BMDL will be selected if the BMDLs estimated from different models varied >3 fold, otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) would be considered appropriate for the data set.

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.4.1) were fit to the data shown in Table B-1 for decreased RBC count and blood hemoglobin concentration in male and female rats.

For RBC counts in male rats, the variance data were not adequately fit by assuming homogenous variance (test 2 *p*-value = 0.05689) or by applying the nonhomogenous variance model (test 3 *p*-value <0.1 shown in Table B-2) in the BMDS, either with all doses included or with the high dose dropped; thus, data sets for RBC counts in male rats were considered not suitable for estimating BMD (Table B-2).

| Table B-1. Red Blood Cell Counts and Blood Hemoglobin Concentrations in Crj:F344 Rats Exposed to Oral 3-Nitroaniline for 28 Days[a] | | | | |
|---|---|---|---|---|
| | Exposure Group (mg/kg-day) | | | |
| Parameter | 0 | 15 | 50 | 170 |
| **Males** | | | | |
| RBC ($10^4$/µL) | 934 ± 39.2 [a] | 868 ± 12.0 [b] | 781 ± 16.8 [b] | 494 ± 27.0 [b] |
| Hgb (g/dL) | 15.6 ± 0.23 | 14.5 ± 0.21 [b] | 14.4 ± 0.31 [b] | 11.8 ± 0.52 [b] |
| **Females** | | | | |
| RBC ($10^4$/µL) | 930 ± 47.2 | 858 ± 31.3 [b] | 769 ± 18.5 [b] | 480 ± 15.2 [b] |
| Hgb (g/dL) | 15.8 ± 0.67 | 14.7 ± 0.40 [b] | 14.4 ± 0.31 [b] | 11.1 ± 0.37 [b] |

[a]Onodera, ND
[b]Means ± SD, sample size = 5/sex/group
[c]Significantly different from control ($p \leq 0.01$)

| Table B-2. Model Predictions for Changes in RBC Count ($10^4$/µL) in Male Rats Exposed to Oral 3-Nitroaniline for 28 Days[a] | | | | | |
|---|---|---|---|---|---|
| Model | Variance model *p*-value[b] | Mean model *p*-value[b] | AIC[b] for fitted model | $BMD_{1sd}$ (mg/kg-day) | $BMDL_{1sd}$ (mg/kg-day) |
| **All dose groups** | | | | | |
| Linear | 0.02434 | 0.1168 | 157.97 | 11.0 | 8.1 |
| Polynomial | 0.02434 | 0.183 | 157.45 | 8.1 | 5.7 |
| Power | 0.02434 | 0.1168 | 157.97 | 11.0 | 8.1 |
| Hill | 0.02434 | 0.1948 | 157.36 | 7.9 | N/A |
| **High dose dropped** | | | | | |
| Linear | 0.05915 | 0.095 | 115.75 | 10.9 | 7.3 |
| Polynomial | 0.05915 | N/A | 114.96 | 5.9 | 3.6 |
| Power | 0.05915 | 0.095 | 115.75 | 10.9 | 7.3 |
| Hill | Failed | Failed | Failed | Failed | Failed |

[a]Onodera, ND
[b]*p*-values from test 3 (nonhomogenous variance model) <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; NA = not available (BMD software could not generate a model output)

For blood hemoglobin concentration data in male rats modeled with all doses included, variance data were fit adequately by the homogenous variance ($p = 0.1417$), therefore, all the models were run with homogenous variance setting (Table B-3). Based on the goodness of fit *p*-values (mean model *p*-value), none of the available models provided adequate fit to the mean response. In order to achieve model fit, the high-dose group was dropped from the analysis.

**Table B-3. Model Predictions for Changes in Blood Hemoglobin Concentration (g/dL) in Male Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Model | Variance model *p*-value[b] | Mean model *p*-value[b] | AIC for fitted model | $BMD_{1sd}$ (mg/kg-day) | $BMDL_{1sd}$ (mg/kg-day) |
|---|---|---|---|---|---|
| **All dose groups** | | | | | |
| Linear | 0.1417 | 0.00136 | -8.37 | 20.7 | 16.0 |
| Polynomial | 0.1417 | 0.00028 | -6.42 | 19.3 | 12.2 |
| Power | 0.1417 | 0.00136 | -8.37 | 20.7 | 16.0 |
| Hill | 0.1417 | 0.00029 | -6.43 | 19.0 | 11.2 |
| **High dose dropped** | | | | | |
| Linear | 0.648 | <0.0001 | -5.84 | 20.2 | 13.3 |
| Polynomial | 0.648 | <0.0001 | -5.84 | 20.2 | 13.3 |
| Power | 0.648 | <0.0001 | -5.84 | 20.2 | 13.3 |
| Hill | failed | failed | failed | failed | failed |

[a]Onodera, ND
[b]*p*-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; NA = not available (BMD software could not generate a model output)

With the reduced data set, the homogenous variance models again fit the variance data adequately. However, none of the available continuous variable models adequately fit the means, as shown in Table B-3 (there were not enough dose groups to apply the Hill model). Thus, data sets for blood hemoglobin concentration in male rats were considered not suitable for BMD modeling.

For RBC counts in female rats modeled with all dose groups included, variance was not adequately fit assuming homogenous variance ($p < 0.1$), but the variance data were adequately fit ($p = 0.3517$) by applying the nonhomogenous variance model in the BMDS (Table B-4). Therefore, BMD modeling results with only nonhomogenous variance models were summarized in Table B-4. Adequate fit for RBC count data were obtained with all four models available in the BMDS ($p > 0.1$), however, the Hill model failed to estimate BMDL (Table B-4). Among Linear, Polynomial and Power models, estimated BMDLs were within 3-fold difference. Since the Polynomial model resulted in the lowest AIC, this model was considered the best model, and the corresponding BMDL of 7.5 mg/kg-day was considered the appropriate BMDL for this end point (Figure B-1).

| Table B-4. Model Predictions for Changes in RBC Count ($10^4$/µL) in Female Rats Exposed to Oral 3-Nitroaniline for 28 Days[a] | | | | | |
|---|---|---|---|---|---|
| **Model** | **Variance model *p*-value[b]** | **Mean model *p*-value[b]** | **AIC for fitted model** | **$BMD_{1sd}$ (mg/kg-day)** | **$BMDL_{1sd}$ (mg/kg-day)** |
| **All dose groups** | | | | | |
| Linear | 0.3517 | 0.1303 | 159.07 | 15.0 | 11.1 |
| Polynomial | 0.3517 | 0.2203 | 158.50 | 10.8 | 7.5 |
| Power | 0.3517 | 0.1303 | 159.07 | 15.0 | 11.1 |
| Hill | 0.3517 | 0.2341 | 158.41 | 10.5 | N/A |

[a]Onodera, ND

[b]*p*-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; NA = not available (BMD software could not generate a model output)

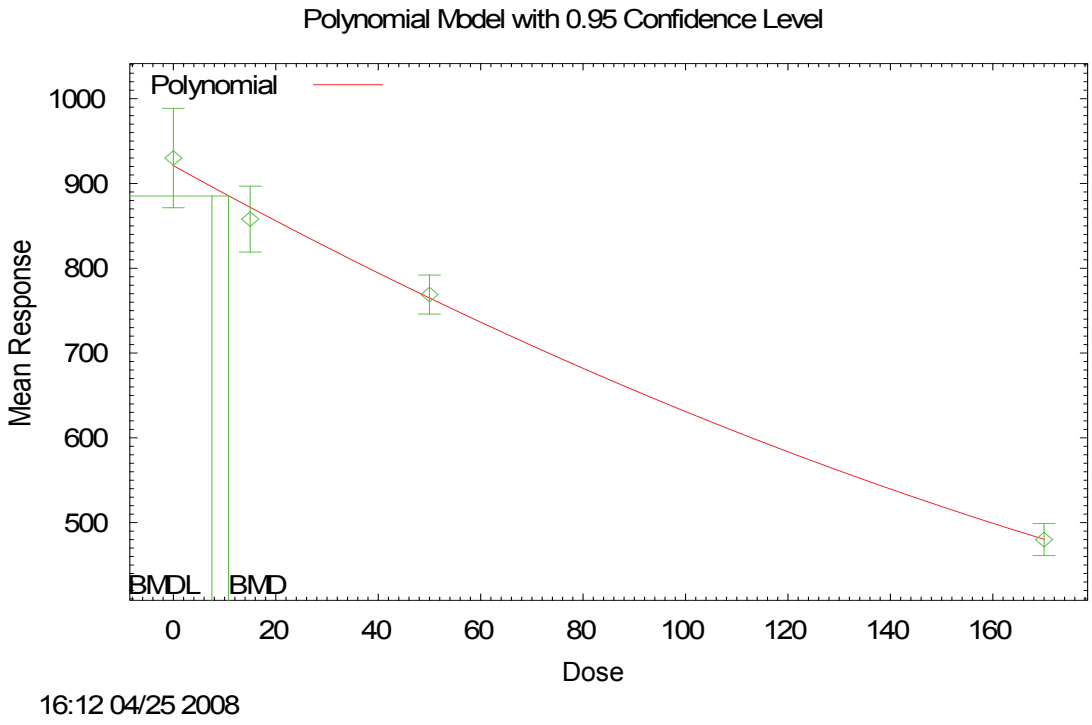


**Figure B-1. Observed and Predicted RBC Counts (104/µL) in Female Rats Exposed to Oral 3-Nitroaniline for 28 Days (High Dose Dropped) (Onodera, ND)**

For blood hemoglobin concentration data in female rats modeled with all doses included, variance data were adequately fit by the homogenous variance ($p = 0.3013$) (Table B-5). With the homogenous variance model applied, none of the available models provided adequate fit to the means (goodness of fit $p$-value <0.1). In order to achieve model fit, the high-dose group was dropped from the analysis. With the reduced data set, the homogenous variance model again fit the variance data adequately ($p = 0.2063$). Assuming homogenous variance, none of the available continuous variable models adequately fit the means, as shown in Table B-5 (there were not enough dose groups to apply the Hill model). Thus, data sets for blood hemoglobin concentration in female rats were considered not suitable for BMD modeling.

**Table B-5. Model Predictions for Changes in Blood Hemoglobin Concentration (g/dL) in Female Rats Exposed to Oral 3-Nitroaniline for 28 Days[a]**

| Model | Variance model *p*-value[b] | Mean model *p*-value[b] | AIC for fitted model | $BMD_{1sd}$ (mg/kg-day) | $BMDL_{1sd}$ (mg/kg-day) |
|---|---|---|---|---|---|
| **All dose groups** | | | | | |
| Linear | 0.3013 | 0.02633 | -2.36 | 19.0 | 14.7 |
| Polynomial | 0.3013 | 0.006998 | -0.35 | 19.1 | 14.7 |
| Power | 0.3013 | 0.02633 | -2.36 | 19.0 | 14.7 |
| Hill | 0.3013 | 0.006991 | -0.36 | 18.9 | 14.6 |
| **High dose dropped** | | | | | |
| Linear | 0.2063 | 0.01228 | 2.20 | 21.8 | 14.1 |
| Polynomial | 0.2063 | 0.01228 | 2.20 | 21.8 | 14.1 |
| Power | 0.2063 | 0.01228 | 2.20 | 21.8 | 14.1 |
| Hill | failed | failed | failed | failed | failed |

[a]Onodera, ND
[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