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Provisional Peer Reviewed Toxicity Values for

p-Chloroaniline
(CASRN 106-47-8)

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 *p*-CHLOROANILINE (CASRN 106-47-8)

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

A chronic oral reference dose (RfD) for *p*-chloroaniline (4-chloroaniline) of 0.004 mg/kg-day, verified in 1987 and available on the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) (U.S. EPA 1988; accessed in 2007), is based on nonneoplastic lesions of the splenic capsule in rats given *p*-chloroaniline in the diet for 78 weeks (NCI, 1979). The LOAEL of 12.5 mg/kg-day was divided by a composite uncertainty factor (UF) of 3000 (10 for extrapolation from a LOAEL to a NOAEL, 10 for extrapolation from rats to humans, 10 to protect sensitive humans and 3 for lack of supporting reproductive and other toxicity data). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not include an RfD for *p*-chloroaniline. The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997) adopted the chronic RfD as the subchronic RfD. Neither IRIS (U.S. EPA, 1988) nor HEAST (U.S. EPA, 1997) list a chronic inhalation reference concentration (RfC), cancer oral slope factor (OSF) or inhalation unit risk (IUR) for *p*-chloroaniline. The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA 1991, 1994) include a Health and Environmental Effects Document (HEED) on chloroanilines (U.S. EPA, 1987) that provisionally assigned *p*-chloroaniline to weight-of-evidence Group C, as a "possible human carcinogen," but declined to derive RfD values due to the potential carcinogenicity. The basis for the Group C designation was suggestive evidence in rodents (in the absence of data in humans): rare splenic tumors in male rats and hemangiosarcomas in male and female mice exposed to *p*-chloroaniline in feed (NCI, 1979) and preliminary results from a gavage study (NTP, 1989) that appeared to confirm the results of the NCI study. A human cancer OSF of 0.035 $(mg/kg\text{-}day)^{-1}$ was calculated in the HEED from the NCI (1979) study.

The International Agency for Research on Cancer (IARC, 1997) assigned *p*-chloroaniline to Group 2B, possibly carcinogenic to humans, based on inadequate evidence in humans and sufficient evidence in animals: increased splenic tumors in male rats and hemangiomas in mice exposed to *p*-chloroaniline in feed (NCI, 1979) or to *p*-chloroaniline hydrochloride (CASRN

20265-96-7) by gavage (NTP, 1989; Chhabra et al., 1991). *p*-Chloroaniline is not included in NTP's 11th Report on Carcinogens (NTP, 2005). Neither the Agency for Toxic Substances and Disease Registry (ATSDR, 2006) nor the World Health Organization (WHO, 2006) has written a toxicological review document for *p*-chloroaniline or for chloroanilines as a group. A toxicity review on aromatic amino compounds and their halogenated derivatives (Woo and Lai, 2001) was among the documents consulted for relevant information.

Literature searches were conducted for studies relevant to the derivation of provisional toxicity values and elucidation of the cancer mode of action for *p*-chloroaniline. The databases TOXLINE Special, PUBMED plus the PUBMED Cancer Subset (replaces CANCERLIT), and DART/ETIC were searched from the 1960s to December, 2006. BIOSIS was searched from December 1999 to December 2006. Searches of RTECS, GENETOX, HSDB, CCRIS, and TSCATS were not date limited. TSCATS2 was searched from January 2002 to December 2006. The Current Contents database was searched from June 2006 to December 2006. An updated search of available literature was performed in PUBMED from January, 2007 to May, 2008.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure

No data were located regarding the subchronic or chronic oral toxicity or carcinogenicity of *p*-chloroaniline in humans.

Inhalation Exposure

Few data were located to describe the toxicity of inhaled *p*-chloroaniline in humans. Workplace air concentrations ranging from 37-90 mg/m^3 were associated with anemia, cyanosis, and increased methemoglobin and sulfhemoglobin levels in 2 of the 6 exposed *p*-chloroaniline production workers (Pacseri et al., 1958, as reported in IPCS, 2003); no further details were given. Another group of 14 *p*-chloroaniline workers exhibited reduced hemoglobin and increased methemoglobin levels (magnitude of changes not reported) following *p*-chloroaniline exposure (intensity and duration not reported) (Monsanto Co., 1986, as reported in IPCS, 2003).

Danish neonates (33 of 415) exposed to *p*-chloroaniline as a breakdown product of chlorohexidine (a component of the humidification solution) in incubators had elevated methemoglobin levels (mean: 19%; range: 6.5-45.5%). No other hematology endpoints were reported. Although the exposure concentrations were not reported, the study authors estimated inhalation exposures of up to 0.3 mg/day from calculations assuming complete breakdown of a 0.02% chlorohexidine solution (IPCS, 2003).

Animal Studies

Oral Exposure

Short-term — NTP (1989) performed a 16-day gavage study of *p*-chloroaniline in rats and mice. Groups of 7-week-old F344/N rats and 8-week-old B6C3F1 mice (5/sex/group) were given deionized water or 25, 50, 100, 200, or 400 mg/kg of *p*-chloroaniline hydrochloride (technical grade *p*-chloroaniline, 99.1% purity; obtained from E.I. DuPont and de Nemours and Company, Inc.) by gavage, 5 days/week for a total of 12 doses over 16 days. All animals were observed twice daily and subjected to necropsy on day 16 of the study. Tissues from two rats of each sex from the control and 100 mg/kg groups were collected and examined microscopically for histopathology. Examined tissues included: adrenal glands, bone marrow, brain, esophagus, colon, gall bladder (in mice), heart, jejunum, kidney, liver, lungs and mainstream bronchi, mandibular and regional lymph nodes, pancreas, parathyroid glands, pituitary gland, prostate/testes or uterus/ovaries, salivary glands, seminal vesicles (in mice), skin, spleen, stomach, thymus, thyroid gland, trachea, urinary bladder, and tissue masses. In addition, the spleen was examined in 2 males and females in the 25 mg/kg group.

All of the rats in the 200 and 400 mg/kg dose groups died within 5 days of the onset of treatment (NTP, 1989). No deaths occurred in the lower dose groups. Clinical signs included lethargy in the 200 and 400 mg/kg-day dose groups, cyanosis in the eyes and extremities (dose groups not specified), and labored breathing in the 25 and 50 mg/kg dose groups. Mean terminal body weights were approximately 20% lower than controls for males in the 100 mg/kg dose group, which was the highest dose group with animals surviving to terminal sacrifice. Terminal body weights were similar to control group values (0-6% difference) in 100 mg/kg females and males and females of the lower dose groups. Enlarged spleens were seen in the 25, 50, and 100 mg/kg dose groups, with sinusoidal splenic congestion and hemosiderin deposition seen in the 100 mg/kg males and females examined microscopically.

As in rats, mice in the 200 and 400 mg/kg dose groups died soon after starting treatment. In contrast to rats, however, deaths (1-2 per group) were also seen in all of the lower dose groups. There were no deaths among the control rats. Cyanosis (blue extremities) was observed in the treated mice (dose groups not specified). In the 25–100 mg/kg dose groups, terminal body weights were similar to control mice (not monitored in higher dose groups due to early mortality). Diffuse splenic congestion and diffuse hemosiderosis of the liver Kupffer cells were observed in the 100 mg/kg males and females examined microscopically.

NCI (1979) carried out a 4-week dose range-finding study for *p*-chloroaniline in rats and mice. A total of 6 groups of 6-week-old Fischer F344 rats (5/sex/group) were offered technical grade *p*-chloroaniline (purity unspecified) in the diet at 0, 70, 145, 315, 680, or 1465 ppm for 4 weeks, while 9 groups of B6C3F1 mice (5/sex/group) were given 0, 255, 550, 1180, 2550, 5500, 8080, 11,830, or 17,380 ppm *p*-chloroaniline in the diet for the same duration. Assuming daily dietary consumption equaling 10% of body weight for rats in a subchronic study, daily *p*-chloroaniline intake for rats can be estimated as 0, 7, 15, 32, 68, or 147 mg/kg-day. Assuming daily dietary consumption of 15% of body weight in mice, doses can be estimated as 0, 38, 83, 177, 383, 825, 1212, 1775, or 2607 mg/kg-day. A 2-week observation period followed exposure.

Body weights and food consumption were recorded twice weekly. At necropsy, unspecified tissue observations were made. All rats survived to necropsy, with no dose-related changes to body weight gain observed. The only reported effect was 100% incidence of enlarged spleen with plaque formation in the 68 and 147 mg/kg-day groups. This lesion was not seen in the controls or 7, 15, and 32 mg/kg-day groups. Thus, 68 mg/kg-day is a LOAEL and 32 mg/kg-day is a NOAEL for splenomegaly in rats following short-term oral exposure. In mice, deaths were recorded only in the 1212 mg/kg-day group (100% of males and females) and 2607 mg/kg-day group (4/5 males but 0/5 females). No deaths were observed in the 1775 mg/kg-day group or in the 38-825 mg/kg-day groups. No cause of death was reported. There were no dose-related changes in body weight gain. Enlarged spleens were seen in 5/10 mice at 1775 mg/kg-day (all of the males) and 5/10 mice at 2607 mg/kg-day (all of the females). Enlarged spleens were not observed in the lower dose groups or in controls.

Subchronic — NTP (1989; Chhabra et al., 1991) conducted a 13-week gavage study of *p*-chloroaniline in rats and mice. Groups of 7-week-old F344/N rats (10/sex/group) were administered 5, 10, 20, 40, or 80 mg/kg of *p*-chloroaniline hydrochloride (technical grade *p*-chloroaniline, 99.1% purity) by oral gavage, 5 days/week, for 13 weeks. Groups of 9-week-old B6C3F1 mice (10/sex/group) were dosed on the same schedule with 7.5, 15, 30, 60, or 120 mg/kg. Groups of control rats and mice (10/sex) received deionized water by gavage. All animals were observed twice daily for clinical signs and mortality. Body weights were recorded weekly. Blood was analyzed for methemoglobin, hemoglobin, leukocytes, lymphocytes, segmented neutrophils, monocytes, eosinophils, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin volume, erythrocytes, and nucleated erythrocytes from all animals at the end of the treatment period. Necropsies were performed on all animals surviving to study termination and animals dying or euthanized before completion of treatment. Weights of the brain, heart, liver, lungs, right kidney, spleen, testes, and thymus were measured. All control and high-dose animals were subjected to microscopic examination of the adrenal glands, bone marrow, brain, esophagus, colon, gall bladder (in mice), heart, ileum, jejunum, rectum, nasal cavity, kidney, liver, lungs and mainstream bronchi, mandibular and regional lymph nodes, pancreas, parathyroid glands, pituitary gland, prostate/testes or uterus/ovaries, salivary glands, seminal vesicles (in mice), skin, spleen, stomach, thymus, thyroid gland, trachea, urinary bladder, and gross tissue lesions. In the lower dose groups, the adrenal glands, bone marrow, kidneys, liver, lungs and bronchi, and stomach tissue in mice, and the nasal cavity and spleen in rats were examined.

All male rats survived to the end of the 13-week treatment period, while one high-dose female died prematurely (cause of death not reported) (NTP, 1989; Chhabra et al., 1991). Terminal body weights of high-dose males and females were 16% ($p < 0.01$) and 4% lower than controls, respectively. Body weights from other groups were not affected by treatment. Significant, dose-related increases in absolute spleen weights were observed in both sexes (see Table 1). Spleen weights were not measured in control males (reasons for the missing spleen weights were not given); therefore, statistical comparisons were made against the low-dose group. In females, a dose-related increase in spleen weights was observed relative to controls.

Table 1. Absolute Spleen Weights (mg) in F344/N Rats Given Gavage Doses of *p*-Chloroaniline for 13 Weeks[a]

Sex	Controls	5 mg/kg	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg
Male	NA[b]	804 ± 16	1057 ± 19	1638 ± 25[c]	3368 ± 54[c]	4748 ± 161[c]
Female	447 ± 13	607 ± 9[d]	806 ± 15[d]	1422 ± 30[d]	2413 ± 71[d]	3527 ± 57[d]

[a]NTP, 1989
[b]NA = Not available; spleens of untreated animals of comparable age reported by NTP to weigh 0.678-0.848 g
[c]Statistically significant ($p < 0.01$), compared to 5 mg/kg group
[d]Statistically significant ($p < 0.01$), compared to controls

All treated groups of both sexes exhibited significant decreases in hematocrit, hemoglobin, and erythrocytes and significant increases in methemoglobin levels (see Table 2). Females also exhibited significant increases in mean cell volume (MCV), leukocytes, and lymphocytes in all treated groups and increases in segmented neutrophils in groups receiving ≥10 mg/kg-day. The study authors noted that the larger magnitude of change in methemoglobin levels in females compared to males may have resulted from delayed blood collection in male rats; blood from males was collected 72 hours after final exposure, compared to 24 hours for females. Hemosiderosis, spleen congestion, and bone marrow hyperplasia were observed in all dose groups of rats treated with *p*-chloroaniline (see Table 3); lesion severity was not reported. Results are consistent with *p*-chloroaniline-induced methemoglobin formation, with subsequent hemolytic anemia and compensatory hematopoiesis. A LOAEL of 5 mg/kg-day, with no associated NOAEL, was identified for hematological effects and splenic lesions indicative of methemoglobinemia and subsequent hemolytic anemia and compensatory hematopoiesis in male and female rats.

In the 13-week NTP (1989) study in mice, seven mice (two 120 mg/kg-day males, three 60 mg/kg-day females, one 30 mg/kg-day female, and one control female) died of pneumonia. No treatment-related changes in body weight were observed. Lymphocytes and leukocytes in males were significantly ($p < 0.05$) depressed in the 7.5 and 15 mg/kg-day groups, respectively, but not in the higher dose groups (see Table 4). Treated groups of both sexes exhibited significant increases in methemoglobin levels at ≥7.5 mg/kg-day, with marked increases in the number of Heinz bodies (denatured hemoglobin inclusions) found in the 30 and 120 mg/kg-day groups (quantitative data not reported), indicating the onset of methemoglobin-induced hemolytic anemia. Decreases in hematocrit and erythrocyte counts and increases in MCV and MCHC indicate anemia and compensatory hematopoiesis.

Body weights were not affected by treatment in either sex. Significant ($p < 0.01$) dose-related increases in spleen weights were observed in all groups of treated males and in females given ≥30 mg/kg-day (see Table 5). In the high-dose groups, the increase in spleen weight versus controls was 6-fold. Other organ weight changes in mice were small (20-25%) increases in heart weight in males given ≥30 mg/kg-day and lung weight in males given ≥60 mg/kg-day, neither of which increased consistently with dose.

Table 2. Hematological Data for the 13-Week Gavage Doses of *p*-Chloroaniline in Male and Female F344/N Rats[a,b]

Parameter	Vehicle Control	5 mg/kg-day	10 mg/kg-day	20 mg/kg-day	40 mg/kg-day	80 mg/kg-day
Males						
Erythrocytes (10^6/mm^3)	9.14 ± 0.06	8.68 ± 0.10[c]	8.18 ± 0.04[c]	7.48 ± 0.06[c]	6.07 ± 0.06[c]	4.90 ± 0.07[c]
Hematocrit (%)	45.5 ± 0.43	42.8 ± 0.49[c]	42.4 ± 0.24[c]	42.7 ± 0.37[c]	39.4 ± 0.31[c]	36.5 ± 0.43[c]
Hemoglobin (g/dL)	15.5 ± 0.11	14.7 ± 0.17[c]	14.6 ± 0.10[c]	15.1 ± 0.13[c]	14.2 ± 0.13[c]	13.4 ± 0.16[c]
Methemoglobin (% of Hgb)	0.08 ± 0.04	0.59 ± 0.10[d]	0.70 ± 0.24[d]	0.68 ± 0.20[d]	0.68 ± 0.19[d]	0.86 ± 0.16[d]
Nucleated erythrocytes (1000/mm^3)	0.00 ± 0.00	1.70 ± 0.50	3.44 ± 0.60[d]	2.90 ± 0.46[d]	8.70 ± 1.36[c]	23.8 ± 1.59[c]
Females						
Erythrocytes (10^6/mm^3)	8.33 ± 0.05	7.77 ± 0.05[c]	7.27 ± 0.08[c]	6.49 ± 0.06[c]	5.69 ± 0.09[c]	5.06 ± 0.06[c]
Hematocrit (%)	45.7 ± 0.26	43.8 ± 0.29[c]	43.3 ± 0.40[c]	42.5 ± 0.34[c]	39.8 ± 0.63[c]	36.3 ± 0.47[c]
Hemoglobin (g/dL)	15.1 ± 0.11	14.4 ± 0.11[c]	14.3 ± 0.14[c]	14.8 ± 0.16[c]	13.7 ± 0.24[c]	13.0 ± 0.12[c]
MCV (microns^3)	55.0 ± 0.00	56.3 ± 0.15[c]	59.3 ± 0.15[c]	65.1 ± 0.23[c]	69.9 ± 0.28[c]	72.2 ± 0.22[c]
Methemoglobin (% of Hgb)	0.46 ± 0.13	1.35 ± 0.15[c]	1.85 ± 0.18[c]	1.73 ± 0.21[c]	2.40 ± 0.15[c]	3.68 ± 0.45[c]
Leukocytes (1000/mm^3)	4.57 ± 0.32	6.04 ± 0.22[d]	8.09 ± 0.28[c]	9.70 ± 0.55[c]	10.26 ± 0.78[c]	6.49 ± 0.39[c]
Lymphocytes (1000/mm^3)	3.84 ± 0.27	5.14 ± 0.23[d]	6.81 ± 0.23[c]	7.99 ± 0.48[c]	7.93 ± 0.65[c]	5.13 ± .33[c]
Segmented neutrophils (1000/mm^3)	0.68 ± 0.08	0.86 ± 0.08	1.17 ± 0.14[d]	1.64 ± 0.24[c]	2.26 ± 0.24[c]	1.33 ± 0.15[c]

[a]NTP, 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.01$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.05$) in William's pairwise test versus control

Table 3. Histological Lesion Incidence for the 13-Week Gavage Doses of *p*-Chloroaniline in Male and Female F344/N Rats[a,b]

Lesion	Vehicle Control	5 mg/kg-day	10 mg/kg-day	20 mg/kg-day	40 mg/kg-day	80 mg/kg-day
Males						
Femoral bone marrow hyperplasia	0/10[c]	10/10	10/10	10/10	10/10	10/10
Spleen hemosiderosis	0/10[c]	10/10	9/10	9/10	4/10	10/10
Spleen congestion	0/10[c]	10/10	10/10	10/10	10/10	10/10
Females						
Femoral bone marrow hyperplasia	0/10[c]	9/10	10/10	10/10	10/10	10/10
Spleen hemosiderosis	0/10[c]	10/10	10/10	8/10	10/10	9/10
Spleen congestion	0/10[c]	10/10	10/10	9/10	10/10	10/10

[a] NTP, 1989
[b] Number of rats with lesion/number of rats examined
[c] All treated groups were statistically significant ($p < 0.05$) in Fisher's exact pairwise test versus control

Table 4. Hematological Data for the 13-Week Gavage Doses of *p*-Chloroaniline in Male and Female B6C3F1 Mice[a,b]

Parameter	Vehicle Control	7.5 mg/kg-day	15 mg/kg-day	30 mg/kg-day	60 mg/kg-day	120 mg/kg-day
Males						
Erythrocytes (10^6/mm^3)	10.66 ± 0.07	10.24 ± 0.13	9.79 ± 0.04[d]	9.26 ± 0.10[d]	8.78 ± 0.09[d]	6.86 ± 0.16[d]
Hematocrit (%)	48.7 ± 0.40	46.9 ± 0.54	45.5 ± 1.34[d]	43.8 ± 0.53[d]	40.4 ± 0.40[d]	32.6 ± 0.82[d]
MCH (pg)	15.7 ± 0.04	15.5 ± 0.18	16.5 ± 0.25[c]	17.6 ± 0.25[d]	20.9 ± 0.29[d]	25.1 ± 0.21[d]
MCHC (g/dl)	34.2 ± 0.09	34.0 ± 0.15	35.4 ± 0.53	37.3 ± 0.39[c]	45.3 ± 0.71[c]	52.6 ± 0.65[c]
Methemoglobin (%)	0.63 ± 0.08	1.72 ± 0.19[d]	1.77 ± 0.14[d]	2.36 ± 0.17[d]	2.84 ± 0.39[d]	3.80 ± 0.20[d]
Leukocytes (1000/mm^3)	8.27 ± 0.52	6.92 ± 0.53	5.44 ± 0.70[b]	7.15 ± 0.75	9.26 ± 0.80	8.99 ± 1.28
Lymphocytes (1000/mm^3)	6.01 ± 0.35	3.76 ± 0.21[c]	1.46 ± 0.34[c]	2.07 ± 0.29	3.10 ± 0.50	3.35 ± 1.01
Females						
Erythrocytes (10^6/mm^3)	10.69 ± 0.15	10.3 ± 0.16	10.18 ± 0.15[c]	9.63 ± 0.10[d]	8.93 ± 0.33[d]	7.26 ± 0.15[d]
Hematocrit (%)	49.8 ± 0.72	47.3 ± 0.75[c]	47.2 ± 0.47[c]	45.67 ± 0.53[d]	41.43 ± 1.46[d]	35.4 ± 0.65[d]
MCH (pg)	15.7 ± 0.07	15.7 ± 0.08	16.3 ± 0.13	17.8 ± 0.17[d]	22.0 ± 0.59[d]	25.0 ± 0.43[d]
MCHC (g/dl)	33.9 ± 0.16	34.1 ± 0.13	35.1 ± 0.19	37.5 ± 0.36[d]	47.2 ± 1.12[d]	51.3 ± 0.80[d]
Methemoglobin (%)	0.29 ± 0.71	0.30 ± 0.11	1.65 ± 0.19[d]	2.88 ± 0.36[d]	3.22 ± 0.15[d]	3.32 ± 0.26[d]

[a]NTP, 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.01$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.05$) in William's pairwise test versus control

Table 5. Absolute organ weights (mg) in B6C3F1 mice given gavage doses of *p*-Chloroaniline for 13 weeks[a,b]

Tissue	Controls	7.5 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Males						
Heart	157 ± 10.2	163 ± 6.2	170 ± 7.0	192 ± 10.0[d]	188 ± 9.8[d]	190 ± 9.2[d]
Lung	228 ± 12	253 ± 12	268 ± 18	264 ± 14	279 ± 15[d]	287 ± 12[c]
Spleen	69 ± 4	107 ± 5[d]	132 ± 21[c]	196 ± 12[c]	266 ± 10[c]	398 ± 14[c]
Females						
Spleen	93 ± 5	97 ± 5	125 ± 6	206 ± 14[c]	293 ± 17[c]	532 ± 24[c]

[a]NTP, 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.01$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.05$) in William's pairwise test versus control

Hematopoiesis in the spleen of both sexes was the most sensitive histological lesion observed, followed by liver and kidney hemosiderosis (see Table 6). Incidence rates for splenic hematopoiesis were ≥80% in all treated groups of males and females. As was observed in rats, increased spleen weight, changes in hematological endpoints, and histological data in mice suggest the onset of methemoglobin-induced hemolytic anemia followed by compensatory hematopoiesis. A LOAEL of 7.5 mg/kg-day was identified in mice exposed to oral *p*-chloroaniline for 13 weeks for increased levels of methemoglobin, changes in hematological parameters, and increased hematopoiesis; a NOAEL was not identified.

Table 6. Histological Lesion Incidence for the 13-Week Gavage Doses of *p*-Chloroaniline in Male and Female B6C3F1 Mice[a,b]

Lesion	Vehicle Control	7.5 mg/kg-day	15 mg/kg-day	30 mg/kg-day	60 mg/kg-day	120 mg/kg-day
Males						
Kidney hemosiderosis	0/10	0/10	0/10	0/10	0/10	9/10[c]
Liver hemosiderosis	0/10	0/10	0/10	2/10	10/10[c]	9/10[c]
Spleen hematopoiesis	0/10	8/10[c]	10/10[c]	10/10[c]	10/10[c]	8/10[c]
Females						
Kidney hemosiderosis	0/10	0/10	0/10	0/10	4/10[c]	10/10[c]
Liver hemosiderosis	0/10	0/10	0/10	0/10	7/10[c]	10/10[c]
Spleen hematopoiesis	2/10	9/10[c]	10/10[c]	9/10[c]	8/10[c]	10/10[c]

[a]NTP, 1989
[b]Number of rats with lesion/number of rats examined
[c]Statistically significant ($p < 0.05$) in Fisher's exact pairwise test versus control

Scott and Eccleston (1967) conducted a 3-month oral *p*-chloroaniline gavage study in rats and dogs. Groups of Wistar rats (10/sex/group) were given daily *p*-chloroaniline doses (purity and vehicle unspecified) of 0, 8, 20, or 50 mg/kg-day. Beagle dogs (4/sex/group) were given 0, 5, 10, or 15 mg/kg-day. Reported hematological endpoints included incidences of changes in hemoglobin, red blood cell (RBC), and Heinz body counts, packed cell volume (PCV), and reticulocytes beyond author-specified thresholds. Data for absolute counts were not reported. Histopathological evaluations were also performed at necropsy, although tissues examined were not specified. Results of statistical analyses, if performed, were not reported.

In rats, both elevated Heinz body counts (>20 per 100 RBCs) and reticulocyte responses (>2%) were observed in 10/10 animals in the high-dose (50 mg/kg-day) groups (Scott and Eccleston, 1967), but 0/10 animals in each of the lower-dose (8 or 20 mg/kg-day) and control groups. In dogs, the incidences of decreased hemoglobin levels, RBC counts, and PCV, and the increased reticulocyte counts and Heinz body counts were dose-related (see Table 7). Statistical significance was achieved for increased incidence of dogs with elevated reticulocyte response and Heinz body counts in mid- and high-dose dogs ($p > 0.05$ using Fisher Exact test, calculated for this review) and for reduction in PCV in high-dose dogs. High-dose rats and all treated groups of dogs exhibited increased incidences of elevated hemosiderin levels and extramedullary

Table 7. Hematological Changes in Beagle Dogs Given Gavage Doses of *p*-Chloroaniline[a,b]

Dose	Hemoglobin reduction (≥2g/100 ml)	RBC reduction (≥1.5M/cm)	PCV reduction (≥10%)	Reticulocyte increase (≥2%)	Heinz body increase (≥20/100 RBCs)
0	0/8	0/8	0/8	0/8	0/8
5	1/8	1/8	1/8	3/8	2/8
10	3/8	2/8	3/8	7/8[c]	6/8[c]
15	4/7	5/7	6/7[c]	7/7[c]	6/7[c]

[a]Scott and Eccleston, 1967
[b]Number of dogs with hematological change/number of dogs examined
[c]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

hematopoiesis in the spleen and liver, as well as bone marrow hyperplasia (incidences not reported). This study identified NOAEL and LOAEL values of 20 and 50 mg/kg-day, respectively, for rats and 5 and 10 mg/kg-day, respectively, for dogs for hematological changes.

Khamuev (1967, as reviewed by IPCS, 2003) gave daily gavage doses of 0 or 37 mg/kg-day in sunflower oil to albino rats (strain, number, and sex unspecified) for 3 months. No other experimental details were reported. Observations include cyanosis, reduced movement, and significantly increased methemoglobin and urobilin levels, spleen weights, and reticulocyte and polychromatic normoblast counts. Significant decreases were observed in hemoglobin levels and erythrocyte counts. Thus, a LOAEL of 37 mg/kg-day was identified for increased methemoglobin and changes in hematopoiesis; a NOAEL was not identified.

Khamuev (1967, as reviewed by IPCS, 2003) also gave daily gavage doses of 0, 0.05, 0.5, and 5 mg/kg-day in sunflower oil to guinea pigs (strain, number, and sex unspecified) for 7 months. No other experimental details were reported. The only effects reported were dystrophic changes in the liver and kidneys (magnitude and direction of change unspecified). These data are inadequate for identification of a NOAEL or LOAEL.

Chronic — NCI (1979) exposed rats to *p*-chloroaniline in the diet for 78 weeks. Groups of Fischer 344 rats (50 per sex per group) were fed diets containing 250 or 500 ppm of technical grade *p*-chloroaniline (purity unspecified) for 78 weeks, followed by a control diet for 24 weeks. Assuming that food consumption in the rat is 5% of body weight for a chronic study, the daily *p*-chloroaniline intakes were calculated as 12.5 mg/kg-day and 25 mg/kg-day for the low- (250 ppm) and high-dosed (500 ppm) rats, respectively. A group of 20 rats/sex were offered the control diet for 102 weeks. Daily clinical observations were made. Body weights were recorded weekly for the first 6 weeks, bi-weekly for the next 12 weeks, and monthly thereafter. No hematological tests, clinical chemistry, or urinalysis were performed. All dead or euthanized animals were necropsied and microscopic examinations were performed on all gross lesions and 28 major tissues.

Survival was significantly reduced ($p < 0.05$) in the high-dose males (76% mortality in high-dose, compared to 10% mortality in controls) but not in the females (10% mortality in high-dose and control groups). Reduced survival in high-dose males first became evident at 60 weeks

and accelerated after 90 weeks. The cause of death was not identified. Body weight gain was not affected in male rats, but it was slightly reduced in high-dose females after week 40 (quantitative data and statistical significance not reported). The incidence of non-neoplastic lesions of the spleen (fibrosis or focal fibrosis of the spleen or splenic capsule) was significantly increased, compared to controls, in both treated groups of males and females (see Table 8). A LOAEL of 12.5 mg/kg-day was identified for focal fibrosis of the splenic capsule in male and female rats. No NOAEL was identified for this species.

Table 8. Incidence of Lesions of the Spleen or Splenic Capsule in the 2-year Feeding Study of *p*-Chloroaniline in Male and Female F344/N Rats[a,b]

	Male Rats			Female Rats		
Lesion	**Control**	**12.5 mg/kg-day**	**25 mg/kg-day**	**Control**	**12.5 mg/kg-day**	**25 mg/kg-day**
Focal fibrosis of splenic capsule	0/20[c]	45/49[d] (92%)	38/49[d] (72%)	0/20[c]	30/48[d] (63%)	43/50[d] (86%)
Fibroma	0/20[c]	0/49	6/49 (12%)	0/20	0/48	0/50
Fibroma or Fibrosarcoma	0/20[c]	0/49	7/49 (14%)	0/20	0/48	0/50
Fibrosarcoma, Hemangiosarcoma, Osteosarcoma or Sarcoma Not Otherwise Specified (NOS)	0/20[c]	0/49	4/49 (8%)	0/20	0/48	1/50
Fibroma, Fibrosarcoma, Hemangiosarcoma, Osteosarcoma or Sarcoma NOS	0/20[c]	0/49	10/49[d] (20%)	0/20	0/48	1/50

[a]NCI, 1979
[b]Number of rats with lesion/number of rats examined, ( ) = incidence expressed as percent
[c]Statistically significant increasing trend in treatment groups versus control ($p < 0.05$) in Cochran-Armitage trend test
[d]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

A significant increase in the incidence of mesenchymal tumors (fibroma, fibrosarcoma, hemangiosarcoma, osteosarcoma, unspecified sarcoma) of the spleen occurred in the high-dose males but not females (see Table 8). The splenic fibromas, fibrosarcomas, osteosarcomas, and hemangiosarcomas were considered treatment-related because their historical incidences were each 0/360 for Fischer 344 rats in this colony. Cochran-Armitage tests conducted by NCI (1979) indicated a significant positive trend in the incidence of individual and combined splenic tumors in males. The results of Fisher exact tests were reported to be significant for combined, but not individual, splenic neoplastic tumor types in males.

NCI (1979) also performed a chronic *p*-chloroaniline feeding study in mice. Groups of B6C3F1 mice (50 per sex per group) were fed diets containing 2500 or 5000 ppm of technical grade *p*-chloroaniline (purity unspecified) for 78 weeks, followed by a control diet for 13-weeks. Assuming that food consumption in the mouse was 15% of body weight, the daily

p-chloroaniline intakes were calculated as 375 mg/kg-day and 750 mg/kg-day for low- and high-dose mice, respectively. A group of 20 mice/sex received the control diet for 91 weeks. Mice were inspected twice daily for clinical signs. Body weights were recorded weekly for the first 6 weeks, bi-weekly for the next 12 weeks, and monthly thereafter. All dead or euthanized animals were necropsied and microscopic examinations were performed on all gross lesions and 29 major tissues.

No treatment-related effects on survival were observed. Of the 11 high-dose females not alive at the study's end, 8 were reported as missing (2 in week 18 and 6 in week 50). Survival in low-dose female mice was reduced compared to control and high-dose females starting at week 60. Terminal survival in this group was approximately 80%, compared with >90% in control and high-dose females. After week 15 and continuing to the end of the study, body weight was depressed in both treatment groups of both sexes, compared to controls. The difference from the controls was approximately 25% for both sexes and both dose groups from week 45 through the end of the study. Moderate-to-heavy iron-positive pigmentation was observed in the liver of treated males and kidney, spleen, and most of the other examined tissues (specific tissues not reported) of both treated sexes. The study authors interpreted the pigmentation to be hemosiderin, indicative of compound-related hemolysis. A LOAEL of 375 mg/kg-day, with no associated NOAEL, was identified for hemolysis in mice.

Tumor incidences were not statistically significant in treated male mice (see Table 9). In females, the Cochran-Armitage test showed significant positive associations ($p < 0.05$) between dose and the incidences of hemangiosarcoma, hemangiosarcoma, or hemangioma combined and hepatocellular carcinoma or hepatocellular adenoma combined. The Fisher exact test results were significant ($p < 0.05$) only for the combined incidence of hemangiosarcoma or hemangioma in high-dose females. Since the incidence of hemangiosarcomas or hemangiomas in treated mice was considerably higher than the 3% incidence in historical controls (8/262 males and 7/260 females), the authors considered the increase to be suggestive of *p*-chloroaniline carcinogenicity in both male and female mice.

A two-year study of *p*-chloroaniline by gavage exposure (NTP, 1989; Chhabra et al., 1991) was performed in rats. Groups of F344/N rats (50/sex/group) were administered 2, 6, or 18 mg/kg of *p*-chloroaniline hydrochloride (technical grade *p*-chloroaniline, 99.1% purity) by gavage, 5 days/week for 103 weeks. Groups of control rats (50/sex) received deionized water by gavage. All animals were observed twice daily for clinical signs. Body weights were recorded once weekly for the first 13 weeks and monthly thereafter. Methemoglobin, hemoglobin, and other hematological measurements were conducted on 12-15 rats of each sex per group at 6, 12, 18, and 24 months. All dead or euthanized animals were necropsied. Microscopic examinations were performed on all gross lesions and on 28 tissues in all high-dose and control animals and in lower-dose animals that died prematurely and on 7 tissues in other animals in the lower-dose groups.

Treatment with *p*-chloroaniline had no adverse effect on survival or body weight. Mid-dose males and high-dose males and females exhibited blue extremities, indicative of cyanosis (time of onset not reported). Beginning at 6 months, hematological changes in the ≥2 mg/kg-day groups of both sexes included reductions in hemoglobin levels, erythrocytes, and hematocrit, and

Table 9. Incidence of Neoplastic Tumors in a 2-year Feeding Study of *p*-Chloroaniline in Male and Female B6C3F1 Mice[a,b]

	Male Mice			Female Mice		
Lesion	**Control**	**375 mg/kg-day**	**750 mg/kg-day**	**Control**	**375 mg/kg-day**	**750 mg/kg-day**
Hemangiosarcoma (all sites)	2/20 (10%)	9/50 (18%)	14/50 (28%)	0/18[c]	3/49 (6%)	7/42 (17%)
Hemangiosarcoma or Hemangioma (all sites)	2/20 (10%)	10/50 (20%)	14/50 (28%)	0/18[c]	3/49 (6%)	8/42[d] (19%)
Hepatocellular Carcinoma	1/19 (5%)	3/49 (6%)	1/49 (2%)	0/18	0/49	3/41 (7%)
Hepatocellular Carcinoma or Adenoma	3/19 (16%)	7/49 (14%)	2/49 (4%)	0/18[c]	1/49 (2%)	6/41 (15%)

[a]NCI, 1979
[b]Number of rats with lesion/number of rats examined, ( ) = incidence expressed as percent
[c]Statistically significant increasing trend in treatment groups versus control ($p < 0.05$) in Cochran-Armitage trend test
[d]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

increases in methemoglobin, MCV, MCH, and nucleated erythrocytes. These findings indicate a compensatory response resulting from hemolytic anemia (see Tables 10 and 11) and corroborate the hematological effects observed in the 13-week studies (NTP, 1989). At 24 months, the differences in hematological values for control and treated groups were much less remarkable than at earlier times, which is likely related to the fact that the 24-month data were collected 11-14 days after the final dose.

Proliferative mesenchymal lesions of the spleen increased in incidence and severity in treated rats (see Table 12). The incidence of splenic fibrosis (nonneoplastic fibrous connective tissue) was dose-related and significantly increased in all treated male groups and in high-dose females; stromal metaplasia was also significantly higher in the high-dose group in both sexes. In the adrenal medulla, a dose-related and significant increase in incidence of hyperplasia was observed in the high-dose females but not in males (see Table 13). For rats, the low dose of 2 mg/kg-day was a LOAEL for increased incidence of splenic fibrosis and hematological effects. No NOAEL was identified.

A significant positive trend was observed for splenic fibrosarcomas and osteosarcomas in males, although the incidences of these tumors were significantly elevated in pairwise tests in high-dose males only (see Table 12). No rat had both fibrosarcoma and osteosarcoma of the spleen. Although tumor incidences did not exhibit a positive trend and were not significantly different in control and treated females, the splenic fibrosarcomas and osteosarcomas observed in single mid-dose and high-dose females, respectively, were considered by the researchers to arise from *p*-chloroaniline exposure since, historically, splenic fibro- or osteosarcomas had never been observed in NTP (1989) water gavage controls (0/297) or untreated controls (0/1961). In the adrenal medulla, a positive trend and increased incidence of adrenal pheochromocytomas was

Table 10. Hematological Effects in Male Fischer F344/N Rats Given Gavage Doses of *p*-Chloroaniline for up to 2 Years[a,b]

Hematological Parameter	0 mg/kg-day	2 mg/kg-day	6 mg/kg-day	18 mg/kg-day
Hemoglobin (g/dL)				
6 months	15.3 ± 0.21	15.2 ± 0.27	14.6 ± 0.14[c]	14.7 ± 0.14[c]
12 months	14.3 ± 0.28	14.5 ± 0.16	13.9 ± 0.28	14.4 ± 0.18
18 months	14.4 ± 0.19	13.8 ± 0.42	13.9 ± 0.24	13.7 ± 0.47
24 months	14.1 ± 0.41	14.0 ± 0.33	13.9 ± 0.48	13.8 ± 0.71
Hematocrit (%)				
6 months	45 ± 0.5	44 ± 1.1	42 ± 0.5[d]	41± 0.4[d]
12 months	42 ± 0.9	43 ± 0.5	42 ± 0.1	43 ± 0.5
18 months	47 ± 0.7	46 ± 1.3	45 ± 0.8	43 ± 1.3[c]
24 months	42 ± 0.41	42 ± 0.9	42 ± 1.4	40 ± 2.1
Erythrocytes (10^6/mm^3)				
6 months	9.34 ± 0.10	9.10 ± 0.19	8.61 ± 0.09[d]	7.58 ± 0.06[d]
12 months	8.91 ± 0.18	9.04 ± 0.10	8.57 ± 0.19	8.38 ± 0.10[c]
18 months	8.92 ± 0.11	8.89 ± 0.18	8.47 ± 0.17	7.32 ± 0.21[d]
24 months	7.82 ± 0.27	8.07 ± 0.18	8.08 ± 0.26	7.54 ± 0.40
MCV (μ^3)				
6 months	48 ± 0.2	49 ± 0.2[c]	50 ± 0.3[d]	54 ± 0.2[d]
12 months	48 ± 0.3	47 ± 0.2	49 ± 0.3[d]	51 ± 0.2[d]
18 months	53 ± 0.6	51 ± 0.9	54 ± 0.5	58 ± 0.5[d]
24 months	54 ± 0.7	52 ± 0.5	52 ± 0.5	54 ± 0.6
Nucleated erythrocytes (/100 leukocytes)				
6 months	1 ± 0.3	1 ± 0.5	2 ± 0.4	4 ± 0.3[d]
12 months	1 ± 0.2	1 ± 0.3	2 ± 0.5	3 ± 0.4[d]
18 months	1 ± 0.3	1 ± 0.4	2 ± 0.4	5 ± 1.5[d]
24 months	3 ± 0.7	1 ± 0.2	1 ± 0.4	4 ± 1.1
MCH (pg)				
6 months	16.4 ± 0.09	16.7 ± 0.14	16.9 ± 0.1[d]	19.4 ± 0.10[d]
12 months	16.1 ± 0.15	16.0 ± 0.09	16.2 ± 0.12	17.2 ± 0.09[d]
18 months	16.1 ± 0.17	15.5 ± 0.26	16.4 ± 0.18	18.7 ± 0.36[d]
24 months	18.1 ± 0.22	17.3 ± 0.20[c]	17.2 ± 0.20[d]	18.4 ± 0.19
MCHC (%)				
6 months	34.4 ± 0.16	34.2 ± 0.36	34.3 ± 0.29	36.0 ± 0.19[d]
12 months	33.7 ± 0.29	33.9 ± 0.15	33.3 ± 0.17	33.4 ± 0.14
18 months	30.5 ± 0.40	30.3 ± 0.19	30.7 ± 0.11	32.1 ± 0.44[d]
24 months	33.6 ± 0.21	33.2 ± 0.27	33.2 ± 0.23	34.1 ± 0.27

Table 10. Hematological Effects in Male Fischer F344/N Rats Given Gavage Doses of *p*-Chloroaniline for up to 2 Years[a,b] (continued)

Hematological Parameter	0 mg/kg-day	2 mg/kg-day	6 mg/kg-day	18 mg/kg-day
Methemoglobin (%hemoglobin)				
6 months	0.26 ± 0.11	0.79 ± 0.15[c]	0.89 ± 0.18[d]	1.97 ± 0.17[d]
12 months	0.28 ± 0.06	0.41 ± 0.09	1.08 ± 0.12[d]	1.18 ± 0.17[d]
18 months	1.04 ± 0.06	1.96 ± 0.13[d]	2.37 ± 0.25[d]	4.09 ± 0.25[d]
24 months	1.56 ± 1.33	1.79 ± 0.14	2.16 ± 0.10[d]	2.17 ± 0.20[d]
Leukocytes (1000mm^3)				
6 months	6.0 ± 0.37	5.9 ± 0.36	7.1 ± 0.38	6.7 ± 0.21
12 months	5.0 ± 0.27	6.0 ± 0.24	7.0 ± 0.58[d]	8.6 ± 0.56[d]
18 months	5.2 ± 0.31	4.4 ± 0.21	5.0 ± 0.35	8.4 ± 0.72[d]
24 months	6.9 ± 1.07	5.4 ± 0.34	6.1 ± 0.96	8.8 ± 0.97

[a]NTP, 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.05$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.01$) in William's pairwise test versus control

Table 11. Hematological Effects in Female Fischer F344/N Rats Given Gavage Doses of *p*-Chloroaniline for up to 2 Years[a,b]

Hematological parameter	0 mg/kg-day	2 mg/kg-day	6 mg/kg-day	18 mg/kg-day
Hemoglobin (g/dL)				
6 months	14.8 ± 0.24	14.3 ± 0.18	13.9 ± 0.12[c]	13.2 ± 0.32[c]
12 months	14.8 ± 0.31	14.5 ± 0.19	13.8 ± 0.36	14.8 ± 0.30
18 months	14.6 ± 0.27	14.1 ± 0.18	13.5 ± 0.23[c]	13.5 ± 0.24[c]
24 months	13.2 ± 0.41	13.2 ± 0.43	13.8 ± 0.16	14.1 ± 0.54
Hematocrit (%)				
6 months	45 ± 0.6	43 ± 0.6[d]	43 ± 0.3[d]	40 ± 0.8[c]
12 months	45 ± 1.1	44 ± 0.9	42 ± 1.1	47 ± 1.1
18 months	47 ± 0.7	46 ± 0.6	44 ± 0.7[c]	44 ± 0.7[c]
24 months	39 ± 1.1	40 ± 1.6	41 ± 0.5	41 ± 1.6
Erythrocytes (10^6/mm^3)				
6 months	8.54 ± 0.12	8.08 ± 0.11[c]	7.60 ± 0.06[c]	6.49 ± 0.12[c]
12 months	8.41 ± 0.20	8.13 ± 0.17	7.62 ± 0.20	8.17 ± 0.17
18 months	8.30 ± 0.12	8.00 ± 0.10	7.35 ± 0.12[c]	6.80 ± 0.08[c]
24 months	7.12 ± 0.25	7.22 ± 0.23	7.33 ± 0.16	7.10 ± 0.18
MCV (μ^3)				
6 months	53 ± 0.2	54 ± 0.1[c]	56 ± 0.2[c]	61 ± 0.2[c]
12 months	54 ± 0.2	54 ± 0.2	55 ± 0.3[c]	57 ± 0.2[c]
18 months	57 ± 0.4	57 ± 0.3	59 ± 0.4[c]	66 ± 0.4[c]
24 months	55 ± 0.6	54 ± 0.3	55 ± 0.7	58 ± 1.1[c]
Nucleated erythrocytes (/100 leukocytes)				
6 months	2 ± 0.4	2 ± 0.5	5 ± 0.8[d]	12 ± 1.4[d]
12 months	1 ± 0.5	2 ± 0.3	3 ± 0.6[c]	5 ± 0.4[c]
18 months	2 ± 0.5	3 ± 0.9	6 ± 0.9[d]	10 ± 1.7[c]
24 months	2 ± 0.5	1 ± 0.2	1 ± 0.3	2 ± 0.5
MCH (pg)				
6 months	17.3 ± 0.13	17.7 ± 0.09[d]	18.3 ± 0.07[c]	20.3 ± 0.19[c]
12 months	17.6 ± 0.16	17.9 ± 0.21	18.1 ± 0.10	18.2 ± 0.34
18 months	17.5 ± 0.17	17.6 ± 0.12	18.3 ± 0.16[c]	19.9 ± 0.24[c]
24 months	18.5 ± 0.19	18.3 ± 0.12	18.7 ± 0.23	19.7 ± 0.40[c]
Reticulocytes (%erythrocytes)				
6 months	NA	NA	NA	NA
12 months	1.5 ± 0.10	1.6 ± 0.12	2.6 ± 0.15[c]	2.7 ± 0.21[c]
18 months	2.5 ± 0.10	2.9 ± 0.21	5.3 ± 0.25[c]	8.4 ± 0.47[c]
24 months	2.5 ± 0.65	2.7 ± 0.84	2.1 ± 0.20	5.1 ± 2.32

Table 11. Hematological Effects in Female Fischer F344/N Rats Given Gavage Doses of *p*-Chloroaniline for up to 2 Years[a,b] (continued)

Hematological parameter	0 mg/kg-day	2 mg/kg-day	6 mg/kg-day	18 mg/kg-day
Methemoglobin (%hemoglobin)				
6 months	0.20 ± 0.11	0.63 ± 0.32	0.07 ± 0.03	0.45 ± 0.14
12 months	0.47 ± 0.11	0.34 ± 0.07	1.16 ± 0.12[c]	1.82 ± 0.12[c]
18 months	0.75 ± 0.82	1.42 ± 0.11[c]	2.52 ± 0.18[c]	3.41 ± 0.16[c]
24 months	1.67 ± 0.10	1.97 ± 0.10	2.03 ± 0.17[d]	1.91 ± 0.15[d]
Leukocytes (1000mm^3)				
6 months	4.1 ± 0.32	3.9 ± 0.18	4.6 ± 0.19	3.9 ± 0.19
12 months	3.0 ± 0.17	3.0 ± 0.17	3.5 ± 0.39	4.7 ± 0.44[c]
18 months	2.5 ± 0.21	2.9 ± 0.13	3.2 ± 0.24[d]	3.8 ± 0.26[c]
24 months	3.4 ± 0.34	3.5 ± 0.33	4.1 ± 0.46	4.3 ± 0.57

NA = Not available
[a]NTP 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.01$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.05$) in William's pairwise test versus control

Table 12. Incidence of Splenic Lesions in the 2-Year Gavage Study of *p*-Chloroaniline in Male and Female F344/N Rats[a,b]

	Male Rats				Female Rats			
Lesion	**Vehicle Control**	**2 mg/kg-day**	**6 mg/kg-day**	**18 mg/kg-day**	**Vehicle Control**	**2 mg/kg-day**	**6 mg/kg-day**	**18 mg/kg-day**
Non-Neoplastic Lesion: Fibrosis	3/49[c] (6%)	11/50[d] (22%)	12/50[d] (24%)	41/50[d] (82%)	1/50[c] (2%)	2/50 (4%)	3/50 (6%)	42/50[d] (84%)
Neoplastic Lesions: Hemangiosarcoma	0/49	0/50	0/50	4/50 (8%)	0/50	0/50	0/50	0/50
Fibrosarcoma	0/49[c]	1/50 (2%)	2/50 (4%)	17/50[d] (34%)	0/50	0/50	1/50 (2%)	0/50
Osteosarcoma	0/49[c]	0/50	1/50 (2%)	19/50[d] (38%)	0/50	0/50	0/50	1/50 (2%)
Fibrosarcoma, Osteosarcoma or Hemangioma	0/49[c]	1/50 (2%)	3/50 (6%)	38/50[d] (76%)	0/50	0/50	1/50 (2%)	1/50 (2%)

[a]NTP, 1989
[b]Number of rats with lesion/number of rats examined, ( ) = incidence expressed as percent
[c]Statistically significant increasing trend in treatment groups versus control ($p < 0.05$) in Cochran-Armitage trend test
[d]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

statistically significant in high-dose males but not in females (see Table 13). From these data, the NTP (1989) concluded that there was clear evidence of carcinogenic activity in male F344/N rats, as shown by the significantly increased incidences of uncommon sarcomas of the spleen and of adrenal pheochromocytomas at the high dose and equivocal evidence in female rats, as shown by the presence of uncommon sarcomas of the spleen in two treated females and the slightly increased incidence of pheochromocytomas. The findings of splenic tumors in F344/N male rats in the NTP (1989) study are consistent with the findings in F344/N male rats in the earlier NCI (1979) study.

Table 13. Incidence of Adrenal Medullary Lesions in the 2-Year Gavage Study of *p*-Chloroaniline in Male and Female F344/N Rats[a,b]

	Male Rats				Female Rats			
Lesion	**Vehicle Control**	**2 mg/kg-day**	**6 mg/kg-day**	**18 mg/kg-day**	**Vehicle Control**	**2 mg/kg-day**	**6 mg/kg-day**	**18 mg/kg-day**
Non-Neoplastic Lesion: Hyperplasia	15/49 (31%)	21/48 (44%)	15/48 (31%)	17/49 (35%)	4/50[c] (8%)	4/50 (8%)	7/50 (14%)	24/50[d] (48%)
Neoplastic Lesions: Pheochromocytoma	13/49[c] (27%)	14/48 (29%)	14/48 (29%)	25/49[d] (51%)	2/50 (4%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Malignant Pheochromocytoma	1/49 (2%)	0/48	1/48 (2%)	1/49 (2%)	0/50	0/50	0/50	0/50
Pheochromocytoma or Malignant Pheochromocytoma	13/49[c] (27%)	14/48 (29%)	15/48 (31%)	26/49[d] (53%)	2/50 (4%)	3/50 (6%)	1/50 (2%)	6/50 (12%)

[a]NTP, 1989
[b]Number of rats with lesion/number of rats examined, ( ) = incidence expressed as percent
[c]Statistically significant increasing trend in treatment groups versus control ($p < 0.05$) in Cochran-Armitage trend test
[d]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

NTP (1989; Chhabra et al., 1991) also conducted a 2-year *p*-chloroaniline gavage study in mice. Groups of B6C3F1 mice (50/sex/group) were administered 3, 10, or 30 mg/kg of *p*-chloroaniline hydrochloride (technical grade *p*-chloroaniline, 99.1% purity) by gavage, 5 days/week for 103 weeks. Groups of control mice (50/sex) received deionized water by gavage. All animals were observed twice a day. Body weights were recorded weekly for the first 13 weeks and monthly thereafter. Unlike the rat study, hematological observations were not made for mice. All dead or euthanized animals were necropsied. Microscopic examinations were performed on all gross lesions and on 29 tissues in all high-dose and control mice, in lower-dose mice that died prematurely, and on the liver and spleen in other mice in the lower-dose groups.

Treatment with *p*-chloroaniline adversely affected survival only in mid-dose males after week 99. There was no significant effect on body weight. The only statistically significant ($p < 0.05$) non-neoplastic lesion reported was pigmentation (hemosiderosis) of the Kupffer cells in the liver of 100% and 92% of the high-dose males and females, respectively (see Table 14).

Table 14. Incidence of Lesions in the 2-Year Gavage Study of *p*-Chloroaniline in B6C3F1 Mice[a,b]

	Male Mice				Female Mice			
Lesion	**Vehicle Control**	**3 mg/kg-day**	**10 mg/kg-day**	**30 mg/kg-day**	**Vehicle Control**	**3 mg/kg-day**	**10 mg/kg-day**	**30 mg/kg-day**
Non-Neoplastic Lesion: Pigmentation of Kupffer cells (hemosiderin)	0/50	0/49	0/50	50/50[d] (100%)	0/50	0/50	1/50 (2%)	46/50[d] (92%)
Neoplastic Lesions: Hepatocellular adenoma	9/50 (18%)	15/49 (31%)	10/50 (20%)	4/50 (8%)	0/50	0/50	0/50	0/50
Hepatocellular carcinoma	3/50[c] (6%)	7/49 (14%)	11/50[d] (22%)	17/50[d] (34%)	0/50	0/50	0/50	0/50
Hepatocellular adenoma or carcinoma	11/50 (22%)	21/49[d] (43%)	20/50[d] (40%)	21/50[d] (42%)	6/50 (12%)	9/50 (18%)	8/50 (16%)	11/50 (22%)
Hemangiosarcomas: Hepatic	2/50 (4%)	2/50 (4%)	1/50 (2%)	6/50 (12%)	0/50	0/50	0/50	0/50
Hemangiosarcomas: Splenic	3/50 (6%)	2/50 (4%)	0/50	5/50 (10%)	0/50	0/50	0/50	0/50
Hemangiosarcomas: All sites	4/50[c] (8%)	4/49 (8%)	1/50 (2%)	10/50 (20%)	0/50	0/50	0/50	0/50

[a]NTP, 1989
[b]Number of rats with lesion/number of rats examined, ( ) = incidence expressed as percent
[c]Statistically significant increasing trend in treatment groups versus control ($p < 0.05$) in Cochran-Armitage trend test
[d]Statistically significant ($p < 0.05$) in Fisher exact pairwise test versus control

For mice, NTP (1989) identified a non-cancer LOAEL of 30 mg/kg-day and an associated NOAEL of 10 mg/kg-day for hemosiderosis in the liver.

Male mice were more sensitive to *p*-chloroaniline carcinogenicity than females. The incidence of hepatocellular carcinoma was statistically significant in the mid- and high-dose mice (see Table 14). In addition, the time of first observation of hepatic carcinomas (728 days for controls, 637 days for low-dose group, and 490 days for high-dose group) and the numbers of hepatic carcinomas that had metastasized to the lung in male mice (1/50, 1/49, 2/50, and 9/50) were dose-related. Although there was a significant negative trend for the incidence of hepatocellular adenoma in male mice, the combined incidences of hepatocellular adenoma plus carcinoma was significant in treated males. The incidence of hepatic tumors in female mice was not significant (see Table 14). A positive trend in the incidence of hemangiosarcomas of the liver or spleen was observed in males but not in females. On the basis of these data, the NTP (1989) concluded that there was some evidence for carcinogenicity in male, but not female B6C3F1 mice, as indicated by the increased incidences of hepatocellular neoplasms and of hemangiosarcomas of the liver or spleen.

Inhalation Exposure

An inhalation study of *p*-chloroaniline in rats was conducted by DuPont (1982). Groups of male Crl:CD rats (16/group) were exposed to 0, 12, 53, or 120 mg/m^3, 6 hours/day, 5 days/week for 2 weeks, with a 2-week post-treatment observation period. Rats were observed for clinical signs and weighed daily. Urine samples were collected on days 9 and 13 and analyzed for pH, occult blood, protein, sugar, bilirubin, acetone, and urobilinogen. Blood samples were collected on days 10 and 14 and were analyzed for erythrocytes, hemoglobin, methemoglobin, MCV, MCHC, white blood cells, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, creatinine, total protein, and cholesterol. At necropsy, organ weights were measured for the heart, liver, lung, kidney, spleen, testes, and thymus. Histopathological examinations were performed on the heart, liver, lung, kidney, spleen, testes, thymus, adrenals, brain, stomach, epididymides, esophagus, eyes, parathyroid, skin, bone marrow, trachea, and thymic lymph node. Details of the statistical analyses were not available.

Clinical signs included rales, alopecia, and corneal clouding in the 120 mg/m^3 group. Mild-to-moderate cyanosis was observed during and shortly after exposure to ≥53 mg/m^3. Body weights were lower (quantities not given) than controls in the 120 mg/m^3 group. In the mid- and high-dose groups, the relative spleen weights were 3- and 4-fold higher than controls. Increased methemoglobin levels (12-, 38-, and 65-fold higher than controls for the low-, mid-, and high-dose groups, respectively) and decreased hemoglobin (6%, 14%, and 33% lower, compared to controls, for low-, mid-, and high-dose groups, respectively) were observed in all treated groups. Extramedullary hematopoiesis and hemosiderosis were observed at concentrations ≥12 mg/m^3. A LOAEL of 12 mg/m^3, with no associated NOAEL, was identified for increased methemoglobin and associated effects in rats.

Kondrashov (1969, as reviewed by IPCS, 2003) studied 4-month *p*-chloroaniline inhalation exposures in rats and cats. Rats (19/group; strain and sex not reported) were exposed to 0, 1, or 9.5 mg/m^3 for 4 hours/day, 6 days/week, for 4 months. Cats (8/group; strain and sex not reported) were also exposed for 4 hours/day, 6 days/week, for 4 months to 0, 1, or 6.9 mg/m^3. A 1-month post-exposure observation period was included. No further details were available for experimental methods or statistical analysis. In rats, severe aggression and reduced hemoglobin and erythrocytes occurred in animals treated with ≥1 mg/m^3 for 4 months. In cats, increased Heinz body formation was observed in animals exposed to ≥ 1.04 mg/m^3 beginning at 2 months. No details on magnitude of changes or other findings were available.

Zvezdaj (1970, as reviewed by IPCS, 2003) exposed an unknown number, strain, and sex of rats to 0.15 mg/m^3 *p*-chloroaniline for 3 months and to 0, 1.5, or 15 mg/m^3 for 6 months. No experimental or statistical analysis details were given. *p*-Chloroaniline treatment did not affect body or organ weights. Methemoglobin was 4% in the low-dose group after 2 months and 22% in the high-dose group after 6 months. In the high-dose group, hemoglobin levels were decreased while reticulocytes and Heinz body formation were increased after 6 months. No details on magnitude of reported effect or other observed effects were available.

Other Studies

Genotoxicity Studies

Genotoxicity assays of *p*-chloroaniline in bacteria were primarily negative (see Table 15). *p*-Chloroaniline did not induce mutations in *Salmonella typhimurium* strains TA97, TA1535, TA1537, or TA1538 with or without metabolic activation or in strains TA98 and TA100 without activation and yielded conflicting, but primarily negative, results in the two latter strains with metabolic activation (U.S. EPA, 1987; IARC, 1997; Rashid et al., 1987; Zeiger, 1990). NTP (1989) reported that p-chloroaniline was tested for mutagenicity in *S. typhimurium* by three independent laboratories and noted that mutagenic activity was detected in strain TA98 with metabolic activation by two of the three laboratories and in strain TA100 with metabolic activation by one laboratory only, but was not detected in strains TA97, TA1535, or TA1537. Whereas several independent studies summarized in U.S. EPA (1987) suggested negative mutagenicity in a variety of *S. typhimurium* strains (see Table 6-4 of U.S. EPA, 1987) with or without metabolic activation. With or without metabolic activation, *p*-chloroaniline did not activate *umu* gene expression in *S. typhimurium* TA1535/pSK1002 (*umuC'-'lacZ*) (Sakagami et al., 1988). *p*-Chloroaniline was not mutagenic towards *Escherichia coli* WP2uvrA in tests conducted with or without metabolic activation (U.S. EPA, 1987; IARC, 1997). NTP conducted tests of several structurally related chemicals (aniline, ortho-, and meta-chloroaniline and p-bromoaniline) and found no evidence of mutagenic activity by these chemicals in *S. typhimurium* strains (NTP, 1989).

In cultured L5178Y mouse lymphoma cells, *p*-chloroaniline increased the frequency of forward mutations with or without metabolic activation (U.S. EPA, 1987; Caspary et al., 1988; Wangenheim and Bolcsfoldi, 1988). *p*-Chloroaniline did not induce DNA-strand breaks (alkaline unwinding/hydroxyapatite elution assay) in mouse lymphoma L5178Y/TK$^{+/-}$ (13.7.2 C) cells (Garberg et al., 1988), but did induce DNA single-strand breaks (single-cell alkaline gel electrophoresis, 'Comet' assay) in exfoliated cells isolated from 3 out of 4 human milk samples in assays conducted without metabolic activation (Martin et al., 2000). *p*-Chloroaniline formed covalent bonds to RNA and DNA (relative binding 440/1) in activated but not in inactivated human granulocytes without metabolic activation (Corbett et al., 1989). *p*-Chloroaniline induced cell transformation in cultured C3H/10T1/2 mouse embryo cells and rat embryo cells infected with Rauscher leukemia virus gave mixed results in Syrian hamster embryo cells and negative results with mouse BALB/C 3T3 cells (NTP, 1983; Traul et al., 1981; IARC, 1997; Dunkel et al., 1988). In mice gavaged with 200 mg/kg *p*-chloroaniline, DNA damage was detected ('Comet' assay) within 8 hours in the stomach, bladder, lung, and brain, and, within 24 hours, in the liver and colon; it was not detected in the kidney or bone marrow (Sasaki et al., 1999a,b).

The structurally related chemical, aniline, was reported by NTP (1989) to induce sister chromatid exchanges and chromosomal aberration in vitro in Chinese hamster ovary cells and induce DNA damage (as assayed by alkaline elution) in liver and kidney tissue (but not in spleen tissue) in rats administered 420 mg/kg aniline. However, no DNA damage was noted in these tissues in Swiss mice given the same dose of aniline.

Table 15. Results of *p*-Chloroaniline Genotoxicity Testing

Assay and Test System	Dose/ Concentration Range	HID or LED*	Result	Reference
Reverse mutation in *S. typhimurium* strains TA98, TA100	1-1000 µg/plate; ± S9 activation	1000 (HID)	Negative	Rashid et al., 1987
Reverse mutation in *S. typhimurium* strains TA100	0-2000 µg/plate; ± S9 activation	2000 (HID)	Negative	Zeiger, 1990
Reverse mutation in *S. typhimurium* strains TA97, TA98, TA100, TA1535	0-1,666 µg/plate; ± S9 activation	100 (LED)	Positive (TA98 +S9)	NTP, 1989 (SRI International)
Reverse mutation in *S. typhimurium* strains TA98, TA100	0-2000 µg/plate; ± S9 activation	333 (LED)	Positive (TA98 +S9)	NTP, 1989 (Microbiological Asscoiates)
Reverse mutation in *S. typhimurium* strains TA98, TA100, TA1535, TA1537	0-3,333 µg/plate; ± S9 activation	1000 (HID)	Negative	NTP, 1989 (Case Western Reserve University)
SOS-response in *S. typhimurium* strain TA1535/pSK1002	100-800 µg/mL; ± S9 activation	800 (HID)	Negative	Sakagami et al., 1988
Mutation in *E. coli* WP2uvrA	0.3-3333 µg/plate; ± S9 activation	333 (HID)	Negative	U.S. EPA, 1987; IARC, 1997
Cell transformation in L5178Y mouse lymphoma cells	500-2500 µM; ± metabolic activation	1500 (LED)	Positive	Wangenheim and Bolcsfoldi, 1988
Cell transformation in C3H/10T1/2 mouse embryo cells	0.8-300 µg/mL	0.8 (LED)	Positive	Dunkel et al., 1988
Cell transformation in Rat embryo cells (infected with Rauscher leukemia virus)	14.5 or 19.0 µg/ 5.2 x 10^4 cells	14.5 (LED)	Positive	Traul et al., 1981; NTP, 1983
Cell transformation in BALB/C mouse 3T3 cells	NS	NS	Negative	NTP, 1983
DNA-strand breaks (alkaline unwinding/hydroxyapatite) in L5178Y/$TK^{+/-}$ (13.7.2 C) mouse lymphoma cells	500-3000 µM	3000 (HID)	Negative in the absence of significant cytotoxicity	Garberg et al., 1988
DNA-damage (Comet assay) in exfoliated cells from human milk samples L5178Y mouse lymphoma cells	710 µM	710 (LED)	Positive	Martin et al., 2000
DNA-damage (Comet assay) in mouse stomach, bladder, lung, brain, liver and colon	200 mg/kg (*in vivo* exposure)	200 (LED)	Positive (negative in kidney and bone marrow)	Sasaki et al., 1999a,b

*HID, highest ineffective dose/concentration for negative tests; LED, lowest effective dose/concentration for positive tests; NS, not stated

In conclusion, although negative results have been obtained in some genotoxicity assays, genotoxic activities have been detected in other assays. The available evidence is insufficient to rule out possible genotoxicity from *p*-chloroaniline or structurally related aniline compounds. NTP (1989) concluded that (1) the *in vitro* genotoxic activity of *p*-chloroaniline is most clearly

demonstrated in the presence of metabolic activation and (2) that the potential of *p*-chloroaniline to induce genotoxic effects is supported by the potential reactivity of probable electrophillic metabolic intermediates.

Toxicokinetic Studies

The toxicokinetics of *p*-chloroaniline appear to be similar in humans and animals (rats, mice, and monkeys) (IARC, 1997; NTP, 1989; Woo and Lai, 2001; Neumann, 1988; Ehlhardt and Howbert, 1991). The compound is rapidly and extensively taken up by the gastrointestinal tract; >90% of an administered dose is excreted in the urine over a period of less than 7 days. Estimates of elimination half-life are between 1.5 and 4 hours. *p*-Chloroaniline is metabolized rapidly by the liver, although the extent of first-pass extraction has not been quantified. Metabolites of *p*-chloroaniline are primarily associated with erythrocytes, as hemoglobin adducts (NTP, 1989; Dial et al., 1998). This is consistent with the finding that *p*-chloroaniline has a high affinity for hemoglobin (Neumann, 1988). With repeated exposures, hemoglobin adducts accumulate and eventually reach steady-state levels limited by the average lifetime of erythrocytes, which is 120 days in humans. In rats injected (i.p., s.c., and i.v.) with *p*-chloroaniline, the percentage of injected dose after 3 hours was initially highest in the liver and renal medulla (Dial et al., 1998); at 24 hours, splenic concentrations had increased, presumably as a result of the accumulation of damaged erythrocytes.

The adverse effects of *p*-chloroaniline appear to be dependent upon its bioactivation (IARC, 1997; NTP, 1989; Chhabra et al., 1991). The compound can be oxidized to N-hydroxy-*p*-chloroaniline by cytochrome P450 enzymes in the liver and further oxidized in erythrocytes to *p*-chloronitrosobenzene, which forms sulfinamide adducts with hemoglobin. Electrophilic intermediates of *p*-chloroaniline metabolism could potentially bind with DNA (NTP, 1989). The lack of hepatotoxicity of *p*-chloroaniline in rats is attributed to the ability of the rat liver to reduce N-hydroxy-*p*-chloroaniline to the parent compound (NTP, 1989; Chhabra et al., 1991). An NADH-dependent reductase activity that rapidly converts N-hydroxy-aniline (and 11 other arylamines) to the parent amine has been identified in microsomal fractions from rat and human liver, in primary cultures of F344 rat hepatocytes and the human HepG2 hepatocyte cell line (King et al., 1999). In tests with a different arylamine substrate (N-OH-aminobiphenyl) and its parent compound (4-aminobiphenyl), ten different human microsomal preparations exhibited a rate of N-hydroxy reduction that was greater than the rate of N-hydroxylation by a factor of 2.7- to 55-fold. Reduction rates varied by nearly 7-fold, while N-hydroxylation rates varied by about 13-fold. These findings suggest that the human liver, like the rat liver, may be relatively protected from toxic effects of *p*-chloroaniline.

DERIVATION OF A PROVISIONAL SUBCHRONIC ORAL RfD FOR *p*-CHLOROANILINE

An RfD of 4E-03 mg/kg-day has been derived on IRIS (U.S. EPA 1988; accessed in 2007) based on incidence of splenic nodules in rats (NCI, 1979). In this study, a LOAEL of 250 ppm in the diet was estimated to provide 12.5 mg/kg-day, assuming a food consumption rate of 5% body weight/day. The LOAEL was modified by a combined uncertainty factor of 3000 (10 for extrapolation from a LOAEL to a NOAEL, 10 for interspecies extrapolation, 10 to

protect susceptible human populations and 3 for the absence of supporting reproductive and developmental toxicity data). The RfD was verified in 1987, pre-dating the NTP (1989; Chhabra et al., 1991) subacute, subchronic and chronic oral toxicity, and carcinogenicity studies in rodents.

No human data were available for derivation of a subchronic p-RfD. In animals, a number of subchronic gavage and feeding studies (3-6 months) have consistently identified hematological effects in animals exposed to *p*-chloroaniline (see Table 16). Gavage exposure 5 days/week to duration adjusted doses of 3.6 and 5.4 mg/kg-day in rats and mice, respectively, for 13 weeks (NTP, 1989) and 1.4 mg/kg-day in male rats for 6 months (NTP, 1989) resulted in increased levels of methemoglobin and Heinz bodies and decreased hematocrit, erythrocytes, and lymphocytes, indicating the onset of methemoglobin-induced hemolytic anemia. Concurrent increases in MCV, MCH, nucleated erythrocytes, spleen weight, splenic hematopoiesis and congestion, and hemosiderosis are indicative of compensatory hematopoiesis. Other subacute and subchronic studies in rats, mice, and dogs support these findings. Cyanosis was observed in rats (Khamuev, 1967, as reviewed by IPCS, 2003; NTP, 1989) and mice (NTP, 1989) exposed for ≥16 days. Enlarged spleens and splenic congestion were observed in rats and mice exposed for 16 days (NTP, 1989) or 4 weeks (NCI, 1979). Increased methemoglobin, Heinz body counts, and spleen congestion, and reduced hemoglobin, hemosiderin, hematocrit, RBC counts were seen in rats, mice, and dogs (Khamuev, 1967, as reviewed by IPCS, 2003; Scott and Eccleston, 1967; NCI, 1979; NTP, 1989). Increased reticulocyte response, hematopoiesis, and bone marrow hyperplasia observed in these studies confirm a compensatory response to anemic conditions. Chronic studies have also reported the hematological effects discussed above (NCI, 1979; NTP, 1989) in mice and rats; however, the extent of hemolytic anemia, methemoglobinemia, and compensatory hematopoietic effects was reduced, but not completely reversed, by the end of the studies.

The critical observed effect was increased formation of methemoglobin, leading to anemia, compensatory hematopoiesis, and splenic pathology. This effect was seen in male and female rats given gavage doses of 3.6 mg/kg-day for 13 weeks, male and female mice given 5.4 mg/kg-day for 13 weeks (NTP, 1989), and male rats given 1.4 mg/kg-day for 6 months (NTP, 1989) (duration-adjusted for continuous exposure, see Table 16). Methemoglobin levels in male rats given 1.4 mg/kg-day for 6 months were 3-fold higher than controls. Likewise, methemoglobin levels in male rats given 3.6 mg/kg-day for 13 weeks were more than 7-fold higher than controls, suggesting that the dose-response relationship for the 13-week and 6-month data may be similar. It appears that methemoglobin formation is the primary toxic response from which the other observed responses follow. The ferric iron in methemoglobin, the oxidation product of ferrous iron in normal hemoglobin, cannot bind oxygen. This results in functional anemia and tissue hypoxia. The ferric iron in methemoglobin denatures globulin and forms protein complex precipitates within the RBC, forming Heinz bodies. Heinz body formation and/or hemoglobin precipitation may result in the early splenic removal of RBCs and observed hemolytic anemia (NTP, 1989). Hematopoiesis occurs as a compensatory response to methemoglobin-induced anemia. Thus, increased methemoglobin in rats (NTP, 1989) was identified as the critical effect for derivation of the subchronic p-RfD, with the 6-month interim exposure point of the NTP (1989) chronic study identifying the lowest duration-adjusted LOAEL of 1.4 mg/kg-day for this effect.

Table 16. Summary of Oral Subchronic and Chronic Toxicity Studies of *p*-Chloroaniline in Animals

Reference	Description	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed Responses
Subchronic Studies				
NTP (1989); Chhabra et al. (1991)	An oral gavage study in F344 rats exposed for 6 months (6 months represents an interim sample collection point in a 103 week exposure protocol) Administered doses (mg/kg-day, 5 days/week): 0, 2, 6 or 18 Duration-adjusted average daily doses (mg/kg-day)[a]: 0, 1.4, 4.3 or 12.9	ND	1.4	increased methemoglobin and MCV in males; increased MCH and MCV, and decreased hematocrit in females
NTP (1989); Chhabra et al. (1991)	An oral gavage study in F344/N rats exposed for 13 weeks Administered doses (mg/kg-day, 5 days/week): 0, 5, 10, 20, 40 or 80 Duration-adjusted average daily doses (mg/kg-day)[a]: 0, 3.6, 7.1, 14.2, 28.4 or 56.8	ND	3.6	hematological effects and splenic lesions indicative of methemoglobinemia and subsequent hemolytic anemia and compensatory hematopoiesis in male and female rats

Table 16. Summary of Oral Subchronic and Chronic Toxicity Studies of *p*-Chloroaniline in Animals (continued)

Reference	Description	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed Responses
NTP (1989); Chhabra et al. (1991)	An oral gavage study in B6C3F1 mice exposed for 13 weeks Administered doses (mg/kg-day, 5 days/week): 0, 7.5, 15, 30, 60, or 120 Duration-adjusted average daily doses (mg/kg-day)[a]: 0, 5.4, 10.8, 21.6, 43.2, or 86.4	ND	5.4	increased levels of methemoglobin, changes in hematological parameters and increased hematopoiesis
Scott and Eccleston (1967)	An oral gavage study in Beagle dogs exposed for 3 months Administered doses (mg/kg-day, 7 days/week): 0, 5, 10, or 15	5	10	increased incidence of animals with elevated Heinz body counts and reticulocyte responses
Khamuev (1967, as reviewed by IPCS, 2003)	An oral gavage study in rats (sex and strain not reported) exposed for 3 months Administered doses (mg/kg-day, 7 days/week): 0 or 37	ND	37	increased methemoglobin and reticulocytes, decreased erythocytes and hemoglobin, cyanosis
Scott and Eccleston (1967)	An oral gavage study in Wistar rats exposed for 3 months Administered doses (mg/kg-day, 7 days/week): 0, 8, 20, or 50	20	50	increased incidence of animals with elevated Heinz body counts and reticulocyte responses

Table 16. Summary of Oral Subchronic and Chronic Toxicity Studies of *p*-Chloroaniline in Animals (continued)

Reference	Description	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed Responses
Chronic Studies				
NTP (1989); Chhabra et al. (1991)	An oral gavage study in F344/N rats exposed for 103 weeks Administered doses (mg/kg-day, 5 days/week): 0, 2, 6, or 18 Duration-adjusted average daily doses (mg/kg-day)[a]: 0, 1.4, 4.2, 12.6	Males: ND Females: 4.2	Males: 1.4 Females: 12.6	increased incidence of splenic fibrosis
NTP (1989); Chhabra et al. (1991)	An oral gavage study in B6C3F1 mice exposed for 103 weeks Administered doses (mg/kg-day, 5 days/week): 0, 3, 10, or 30 Duration-adjusted average daily doses (mg/kg-day)[a]: 0, 2.1, 7.1, or 21.4	7.1	21.4	increased incidence of hemosiderosis in the liver
NCI (1979)	A dietary exposure study in F344 rats exposed for 78 weeks, followed by 24 weeks of observation Dietary concentrations: 0, 250, or 500 ppm Estimated doses (mg/kg-day)[b]: Males and females: 0, 12.5, or 25	ND	12.5	focal fibrosis of the splenic capsule

Table 16. Summary of Oral Subchronic and Chronic Toxicity Studies of *p*-Chloroaniline in Animals (continued)

Reference	Description	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed Responses
NCI (1979)	A dietary exposure study in B6C3F1 mice exposed for 78 weeks, followed by 28 weeks of observation Dietary concentrations: 0, 2500, or 5000 ppm Estimated doses (mg/kg-day)[b]: Males and females: 0, 375, or 750	ND	375	hemosiderosis in multiple tissues, reduced body weight

ND = Not determined

[a]Calculated as administered dose x (5 days/7days).

[b]Estimated by assuming consumption of 5% of body weight by rats in a chronic study and 15% by mice.

Data for methemoglobin levels in male and female rats dosed for 13 weeks and male rats dosed for 6 months were initially selected for benchmark dose (BMD) modeling. Since means and variance data were available for this endpoint, the continuous-variable models in the U.S. EPA Benchmark Dose Software (BMDS version 1.4.1) were applied to the data. The calculated BMD_{1SD} and $BMDL_{1SD}$ are estimates of the dose and its 95% lower confidence limit, respectively, associated with a change in the mean methemoglobin levels equal to 1 standard deviation (SD) from the control mean. This benchmark response (BMR) gives an excess risk of approximately 10% for animals having methemoglobin levels above the 98th percentile of controls (U.S. EPA, 2000). BMD modeling was performed using the doses administered in the study, not the duration-adjusted average daily doses calculated in Table 16. Details of the BMD modeling and a plot of the best fitting models, when appropriate, are presented in Appendix A. No adequate model fit could be achieved for the male rat methemoglobin data from the 6-month or the 13-week exposures (NTP, 1989). Methemoglobin data in female rats provided an equivalent adequate fit using the linear, polynomial, and power models, but only after the highest three dose groups were dropped. The BMD_{1SD} and $BMDL_{1SD}$ calculated for the female rat methemoglobin data from the 13-week exposure were 3.39 and 2.55 mg/kg-day, respectively. The 13-week NTP (1989) rat study involved exposure by oral gavage 5 days/week, therefore the BMD_{1SD} and $BMDL_{1SD}$ were duration adjusted to 2.4 and 1.8 mg/kg-day, respectively. However, this duration adjusted $BMDL_{1SD}$ for methemoglobin levels in female rats given *p*-chloroaniline for 13-weeks does not provide a lower point of departure (POD) than the duration adjusted LOAEL ($LOAEL_{ADJ}$) of 1.4 mg/kg-day for male rats exposed for 6 months. As such, a **provisional subchronic RfD of 0.0005 mg/kg-day or 5E-4 mg/kg-day** for *p*-chloroaniline, based on the $LOAEL_{ADJ}$ of 1.4 mg/kg-day as the POD (NTP, 1989), was derived as follows:

subchronic p-RfD	=	$LOAEL_{ADJ} \div UF$
	=	1.4 mg/kg-day ÷ 3000
	=	**0.0005 mg/kg-day or 5E-04 mg/kg-day**

The composite UF of 3000 was composed of the following:

- A 3-fold UF was applied for extrapolation from a LOAEL to a NOAEL; the lowest exposure dose in the critical study, a LOAEL, was identified as the POD. While the potential exists for developing more significant toxicities related to *p*-chloroaniline-induced methemoglobinemia such as hemolytic anemia, organ/tissue hypoxia, and/or splenic lesions, the available subchronic database (primarily the 13-week and 6-month data from NTP, 1989) does not suggest this is the case at doses near the proposed POD. Also, there was a precipitous drop (~ 50% reduction) in methemoglobin levels at the 12-month time point compared to the 6-month time point in male and female rats orally exposed to *p*-chloroaniline at the LOAEL of 1.4 mg/kg-day. This suggests that during a subchronic oral exposure period at doses near the proposed POD, the potential for developing more severe lesions or conditions related to *p*-chloroaniline-induced methemoglobinemia may be less than intuitively predicted. As such, a 3-fold UF was applied for extrapolation from a LOAEL to a NOAEL.

- A 10-fold UF for intraspecies differences was applied 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 *p*-chloroaniline.
- A 10-fold UF for interspecies extrapolation was applied to account for potential pharmacokinetic and pharmacodynamic 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 *p*-chloroaniline-induced methemoglobinemia than rodents.
- A 10-fold UF was included for database deficiencies; while the database includes chronic gavage and feeding studies in two species of animals, no single- or multi-generation reproductive or developmental toxicity studies are available.

The confidence in the critical study is high. The study (NTP, 1989) was a well conducted subchronic duration study. Extensive hematological parameters were evaluated in two species (rats and mice), with the observed dose-response of these parameters suggesting that methemoglobinemia precipitated hemolytic anemia and a compensatory response seen in this study and other subchronic (Khamuev, 1967, as reviewed by IPCS, 2003; Scott and Eccleston, 1967; NCI, 1979) and chronic (NTP, 1989) studies. The confidence in the database is medium, since several well conducted subchronic studies report consistent clinical and hematological findings; however, no data are available for reproductive or developmental effects. Therefore, the confidence in the derived subchronic p-RfD is medium.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfCs FOR *p*-CHLOROANILINE

A limited number of subchronic animal inhalation studies are available for *p*-chloroaniline (DuPont, 1982; Kondrashov, 1969, as reviewed by IPCS 2003; Zvezdaj, 1970, as reviewed by IPCS, 2003). These studies are limited in design and/or reporting detail and are, thus, inadequate for derivation of a subchronic p-RfC. Human case reports of hematological effects (anemia, cyanosis, increased methemoglobin and sulfhemoglobin, and reduced hemoglobin) associated with occupational exposures (Pacseri et al., 1958, as reported in IPCS, 2003; Monsanto Co., 1986, as reported in IPCS, 2003) and accidental exposures of neonates (IPCS, 2003) did not provide sufficient details of study methodology, exposure, and response adequate for the derivation of a subchronic or chronic inhalation RfC for *p*-chloroaniline.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR *p*-CHLOROANILINE

Weight-of-Evidence Descriptor

No data were available for carcinogenic effects of *p*-chloroaniline in humans. Chronic oral bioassays have identified several sites of tumorigenicity in rodents, including splenic

sarcomas in rats (NCI, 1979; NTP, 1989), adrenal tumors in rats (NTP, 1989), and liver tumors in mice (NTP, 1989). The earlier NCI (1979) dietary bioassay yielded suggestive evidence for splenic tumors in rats and hemangiosarcomas in mice. However, carcinogenicity was more definitively demonstrated in the NTP (1989) bioassay, which exposed the animals via gavage administration. NTP (1989; Chhabra et al., 1991) suggested that the relatively weak results in the NCI study may have been influenced by the instability of *p*-chloroaniline in feed, leading to a failure to reach the targeted dosages, and also by the less-than-lifetime exposure duration (78 weeks, followed by observation periods of 24 weeks for rats and 13 weeks for mice).

p-Chloroaniline is genotoxic to a variety of mammalian cells, including *in vitro* cultures of human mammary cells and granulocytes (Martin et al., 2000; Corbett et al., 1989). The non-neoplastic toxicity of *p*-chloroaniline is similar in animals and humans, specifically the generation of methemoglobin. Methemoglobin formation is hypothesized as a precursor step for splenic sarcomas (see the Mode of Action discussion below), suggesting that similar carcinogenic modes of action may be present in humans and animals. The carcinogenic effects of *p*-chloroaniline are similar to those reported for aniline, a structurally related compound listed on IRIS (U.S. EPA 1994; accessed in 2007) as a *B2, probable human carcinogen*. As described on IRIS, rare splenic tumors (fibrosarcoma, stromal sarcoma, capsular sarcoma, and hemangiosarcoma) developed in male CD-F rats that were fed diets containing aniline (CIIT, 1982). Thus, under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), *p*-chloroaniline is classified as "*likely to be carcinogenic to humans*," based on positive tumor development in multiple animal species.

Mode-of-Action Discussion

The U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment define 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. Toxicokinetic processes leading to the formation or distribution of the active agent (i.e., parent material or metabolite) to the target tissue are not part of the mode of action." Examples of possible modes of carcinogenic action include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation and immunologic suppression.

There are no data for *p*-chloroaniline carcinogenicity in humans. Results from rat and mouse studies show that chronic oral exposure to *p*-chloroaniline produced significant increases in the incidence of splenic sarcomas (fibrosarcomas, osteosarcomas, or hemangiosarcomas) in male rats, adrenal tumors (medullary pheochromocytoma or malignant pheochromocytoma) in male rats and liver tumors (hepatocellular carcinoma) in male mice. Chronic oral exposure to structurally related aniline compounds (aniline hydrochloride, azobenzene, D&C Red No. 9, and ortho-toluidine hydrochloride) similarly produced increased incidences of splenic sarcomas or adrenal gland tumors in rats and increased incidences of liver tumors in mice (see NTP, 1989 for review).

Toxic effects of *p*-chloroaniline on the hematopoietic system have been hypothesized to play a role in the mode of action by which *p*-chloroaniline (and other structurally related

aromatic amines including aniline) induces splenic tumors in rats (Bus and Popp, 1987; NTP, 1989). The hypothesized scheme involves the initial formation of a N-hydroxy metabolite in the liver and subsequent transport of the N-hydroxy metabolite to erythrocytes. In the erythrocyte, the N-hydroxy metabolite is oxidized to form methemoglobin and a reactive nitroso metabolite that leads to subsequent erythrocyte cytotoxicity. Damaged erythrocytes are scavenged by the spleen, leading to (1) vascular congestion, hemorrhage, and resultant hyperplasia and fibrosis (potentially involving increased cell proliferation, which could promote the development of spontaneously initiated or chemically-initiated spleen cells into tumors), and (2) the generation of DNA-reactive chemicals in the spleen leading to mutation and the subsequent transformation of splenic cells into initiated tumor cells.

The modes of action by which *p*-chloroaniline and other aniline chemicals induce adrenal tumors in rats and liver tumors in mice has received less research attention than splenic tumors in rats, and detailed mode-of-action hypotheses for p-chloroaniline-induced tumors at these sites have not been developed.

Hypothesized Mutagenic Mode of Action

Information to support a mutagenic mode of action for *p*-chloroaniline includes *p*-chloroaniline-induced mutagenicity in several *in vitro* assays and animal and human cell cultures (U.S. EPA, 1987; IARC, 1997; Rashid et al., 1987; Zeiger, 1990; Sakagami et al., 1988; Dunkel et al., 1988; Caspary et al., 1988; Wangenheim and Bolcsfoldi, 1988; Garberg et al., 1988; Corbett et al., 1989; NTP, 1989; Martin et al., 2000). DNA damage was also detected in mouse livers 24 hours after a single gavage dose of 200 mg/kg (Sasaki et al., 1999a,b). Because this study represents the only available *in vivo* mutagenic study in animals (Sasaki et al., 1999a,b), an assessment could not be made of the dose-response or temporal relationships between genotoxicity and tumor development. It is currently uncertain whether the mode(s) of action by which *p*-chloroaniline induces splenic and adrenal tumors in rats or liver tumors in mice involves a genotoxic key event.

Hypothesized Cell Proliferation-Mediated Mode of Action for Splenic Tumorigenicity

Key Events — The development of splenic tumors in rats may involve a series of events starting with the diffusion of N-hydroxy-*p*-chloroaniline into erythrocytes, where hemoglobin is damaged by the oxidation of heme ferrous iron, forming methemoglobin by covalent binding of N-hydroxy-*p*-chloroaniline to cysteines on the protein chain. The effect of *p*-chloroaniline on the spleen may result from the splenic scavenging of damaged erythrocytes. The increased deposition of erythrocytes and cellular debris are proposed to result in a continuum of observed splenic effects: hemosiderosis, vascular congestion, and hemorrhage, that may lead to stimulation of cell proliferation, which could promote the development of spontaneously initiated spleen cells into tumors, as well as lead to hyperplasia and fibrosis. Structurally related compounds (i.e., aniline and structurally related aromatic amines) have been hypothesized to induce splenic tumors in male rats by a similar mechanism (Bus and Popp, 1987; NTP, 1989).

Strength, Consistency, Specificity of Association — The association between the occurrence of splenic sarcomas and proposed precursor events such as methemoglobin

formation, hemosiderosis, splenic congestion, and splenomegaly is not consistent across rodent species. Rats develop rare splenic sarcomas following chronic oral exposure to p-chloroaniline and other aniline compounds, but mice do not (NTP, 1989); however, with shorter-term (13-week) exposure to *p*-chloroaniline, both rats and mice show methemoglobin formation, extramedullary hematopoiesis, and splenomegaly. Current mechanistic understanding is inadequate to explain why rats develop splenic and adrenal tumors and mice develop liver tumors in response to *p*-chloroaniline, aniline, and other structurally related chemicals.

Dose-Response Concordance — NTP (1989) noted that the dose-response data for splenic tumors in male rats showed evidence of nonlinearity, pointing out that the incidence of high-dose rats with sarcomas was 12 times the incidence of mid-dose rats, whereas the high dose was only 3 times the mid dose. NTP (1989) also noted that evidence of nonlinearity has been reported for the dose-response relationships for splenic tumors in rats exposed to other aniline compounds. Although these observations provide evidence that a similar mode of action may be involved in the induction of splenic tumors by several aniline compounds, evidence for nonlinearity in the dose-response relationship for sarcomas is insufficient to establish that the proposed precursor events (those that might promote spontaneously initiated splenic cells to develop into tumors) are the only key events in the carcinogenic response to these compounds. In addition, the rarity of splenic fibrosarcomas and osteosarcomas in control rats suggests that the tumors observed in the low- and mid-dose exposure groups in the rat NTP (1989) bioassay are likely to have been exposure-related, even though the incidences were not statistically significantly elevated compared with the concurrent control group (1/50 and 3/50 compared with 0/49 in controls).

Dose-response data for methemoglobinemia and non-neoplastic splenic effects indicative of possible cell proliferation events indicate that these effects occur in *p*-chloroaniline-exposed rats and mice beginning at lower exposure levels (2 and 6 mg/kg-day) than those that induced statistically significant increased incidences of splenic sarcomas in male rats (incidences of male rats with fibrosarcomas, osteosarcomas or hemangiosarcomas were 0/49, 1/50, 3/50, and 38/50 for the 0, 2, 6, and 18 mg/kg-day groups, respectively) (NTP, 1989). These observations support the possible involvement of these splenic responses in the carcinogenic response, but they do not establish the responses as key events because similar dose-response relationships were observed in *p*-chloroaniline-exposed mice, which did not develop splenic sarcomas (NTP, 1989).

Temporal Relationships — Methemoglobinemia and splenic effects indicative of enhanced cell proliferation have been observed in rats and mice as early as after 16 days of exposure, whereas splenic tumors have been detected after at least 71 weeks of exposure in rats (NTP, 1989). These observations are consistent with the possibility that hematological and splenic effects (which precede the development of splenic tumors) are involved in the development of *p*-chloroaniline-induced splenic tumors. In the 2-year gavage NTP (1989) bioassay, rats with fibrosarcomas, osteosarcomas, or hemangiosarcomas were first detected after 99, 101, and 71 weeks in the low-, mid-, and high-dose groups, respectively. Rats given ≥25 mg/kg-day for 16 days exhibited enlarged spleens, with splenic congestion seen in rats and mice receiving 100 mg/kg-day group (NTP, 1989). Oral gavage 4-week exposures of ≥34 mg/kg-day in rats and ≥592 mg/kg-day in mice resulted in spleen enlargement (NCI, 1979). A Gavage 13-week exposure of ≥5 mg/kg-day in rats and ≥7.5 mg/kg-day in mice resulted in increased

methemoglobin and splenic hematopoiesis, congestion, and/or hemosiderosis (NTP, 1989). Gavage exposures of 6 months to 2 years of rats to ≥2 mg/kg-day resulted in increased methemoglobin (NTP, 1989).

Biological Plausibility and Coherence — The multi-event hypothesis linking erythrocyte toxicity to the development of non-neoplastic and neoplastic lesions in the spleen is biologically plausible and consistent with a portion of currently available data (e.g., non-neoplastic lesions induced by *p*-chloroaniline temporally precede the development of splenic tumors). However, although key events have not been identified that would explain why both rats and mice show methemoglobin formation and non-neoplastic spleen lesions, rats do develop rare splenic tumors and adrenal gland tumors, and mice develop liver tumors in response to chronic exposure to *p*-chloroaniline.

Conclusions

Currently, available data are insufficient to identify key events in the development of *p*-chloroaniline-induced tumors in rats (splenic and adrenal gland tumors) and mice (liver tumors). Therefore, consistent with U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), a linear (e.g., non-threshold) extrapolation is indicated when a mode of action is not established and/or a threshold for a nonlinear mode of action cannot be identified.

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

There are no human oral data on which to estimate an oral cancer slope factor for *p*-chloroaniline. The tumor incidence data in the NTP (1989) and NCI (1979) studies were used to calculate an OSF for *p*-chloroaniline. Dose-response modeling was performed based on incidence data for splenic tumors in male rats (NTP, 1989; NCI, 1979), adrenal tumors in male rats (NTP, 1989), and hepatocellular carcinoma in male mice (NTP, 1989). Dose-response modeling was performed using methodologies recommended in the 2005 Guidelines for Cancer Risk Assessment (U.S. EPA, 2005) and benchmark dose (BMD) analysis guidance (U.S. EPA, 2000). A default linear extrapolation was used for dose-response modeling.

The incidence data (see Table 17) were analyzed using the multistage-cancer model for dichotomous data in the BMDS program (version 1.4.1) developed by U.S. EPA. Benchmark doses (BMDs) were calculated as doses expected to result in a 10% extra risk for development of each tumor type. Confidence bounds ($BMDL_{10}$) on the estimated BMDs for each tumor type were automatically calculated by the BMDS software using a maximum likelihood profile method.

Details of the BMD modeling are presented in Appendix B. Since the NTP (1989) data were from animals that were dosed for 5 days/week, a duration adjustment was made to each $BMDL_{10}$ from this study by multiplying it by a factor of 5/7 to derive a $BMDL_{10\ ADJ}$. Human equivalents of each animal $BMDL_{10\ ADJ}$ ($BMDL_{10\ HED}$) were derived by correcting for differences in body weight and lifespan between humans and the species tested. U.S. EPA uses a cross-

Table 17. Data Selected for BMD Modeling of Cancer Incidence in Rats and Mice Given Gavage Doses of *p*-Chloroaniline for 78 (NCI, 1979) or 103 (NTP, 1989) Weeks

Study Reference	Tumor Type	Species	Sex	Dose (mg/kg-day)			
				Tumor Incidences			
NCI, 1979	Splenic fibroma, fibrosarcoma, hemangiosarcoma, osteosarcoma, or sarcoma NOS	rat	male	0	12.5	25	
				0/20	0/49	10/49	
NTP, 1989	Splenic fibrosarcoma, Hemangiosarcoma, or osteosarcoma	rat	male	0	2	6	18
				0/49	1/50	3/50	38/50
NTP, 1989	Adrenal medulla pheochromocytoma, or malignant pheochromocytoma	rat	male	0	2	6	18
				13/49	14/48	15/48	26/49
NTP, 1989	Hepatocellular carcinoma	mice	male	0	3	10	30
				3/50	7/49	11/50	17/50

species scaling factor of body weight raised to the ¾ power (U.S. EPA, 1992, 2005). Calculation of the $BMDL_{10\ HED}$s was performed by multiplying the animal BMDL by the ratio of animal to human body weight raised to the ¼ power. The resulting $BMDL_{10\ HED}$s for each tumor type/data set are shown in Table 18.

The lowest $BMDL_{10\ HED}$ was 0.531 mg/kg-day, based on adrenal tumors in rats (NTP, 1989). In particular, pheochromocytomas were observed in the adrenal medulla of rats exposed to oral *p*-chloroaniline for up to two years. These tumors are derived from chromaffin cells of the adrenal gland and interestingly have been shown, *in vitro*, to be sensitive to erythropoietin (Renzi et al., 2002). Mechanistic data demonstrating mitogenic and anti-apoptotic effects of erythropoietin (EPO) on pheochromocytoma cells (e.g. PC12) (Seong et al., 2006; Um et al., 2007) might suggest a potential link between the hematological effects of *p*-chloroaniline (e.g. methemoglobinemia) and the manifestation of an adrenal medulla cancer phenotype. However, there is not sufficient evidence of such a relationship *in vivo*.

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 0.531 mg/kg-day for adrenal medulla tumors in male rats as the POD, **a provisional OSF of 0.2 (mg/kg-day)$^{-1}$** is calculated as follows:

$$\textbf{p-OSF} = 0.1 / BMDL_{10\ HED}$$
$$= 0.1 / 0.531 \text{ mg/kg-day}$$
$$= \textbf{0.2 (mg/kg-day)}^{-1}$$

Table 18. $BMDL_{10}$s and Human Equivalent Doses from Models Adequately Fit to Incidence Data for Tumors in Animals Chronically Treated with *p*-Chloroaniline

Test Group	Study Reference	Tumor Location & Type	$BMDL_{10\ ADJ}$ (mg/kg-day)	$BMDL_{10\ HED}$ (mg/kg-day)
male rat	NCI, 1979	Splenic fibroma, fibrosarcoma, hemangiosarcoma, osteosarcoma or sarcoma NOS	15.084	3.958[b]
male rat	NTP, 1989	Splenic fibrosarcoma, hemangiosarcoma or osteosarcoma	3.027[a]	0.843[c]
male rat	NTP, 1989	Adrenal medulla pheochromocytoma or malignant pheochromocytoma	1.906[a]	0.531[c]
male mice	NTP, 1989	Hepatocellular carcinoma	3.952[a]	0.607[d]

[a]Animal $BMDL_{10\ ADJ}$: $BMDL_{10}$s from Tables B-2 to B-4, adjusted for continuous exposure by multiplying by a factor of 0.714 (5 days/7 days).
[b]$BMDL_{10\ HED}$: Human equivalent $BMDL_{10}$ calculated as (rat BMDL) x $(W_{rat}/W_{human})^{1/4}$, where W_{human} = 70kg (reference human body weight), W_{rat} = 0.332kg (time-weighted average rat body weight in study).
[c]$BMDL_{10\ HED}$: Human equivalent $BMDL_{10}$ calculated as (rat BMDL) x $(W_{rat}/W_{human})^{1/4}$, where W_{human} = 70kg (reference human body weight), W_{rat} = 0.42kg (time-weighted average rat body weight in study).
[d]$BMDL_{10\ HED}$: Human equivalent $BMDL_{10}$ calculated as (mouse BMDL) x $(W_{mouse}/W_{human})^{1/4}$, where W_{human} = 70kg (reference human body weight), W_{mouse} = 0.039kg (time-weighted average mouse body weight in study).

The OSF for *p*-chloroaniline should not be used with exposures exceeding the point of departure ($BMDL_{10\ HED}$ = 0.531 mg/kg-day) because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of *p*-chloroaniline.

Inhalation Exposure

There are no human or animal inhalation data on which to base an inhalation unit risk for *p*-chloroaniline.

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APPENDIX A. DETAILS OF BMD ANALYSIS FOR THE SUBCHRONIC ORAL p-RfD FOR *p*-CHLOROANILINE

Continuous Data

Available continuous-variable models in the U.S. EPA BMDS (linear, polynomial, power, and Hill models; BMDS version 1.4.1) were fit to the data shown in Table A-1 for changes in serum methemoglobin levels in male rats given gavage doses of *p*-chloroaniline for 6 months and the data shown in Table A-2 for serum methemoglobin levels in male and female rats given gavage doses of *p*-chloroaniline for 13 weeks (NTP, 1989).

Table A-1. Methemoglobin Levels in Male F344/N Rats Given Gavage Doses of *p*-Chloroaniline for 6 Months[a]

Endpoint	0 mg/kg-day	2 mg/kg-day	6 mg/kg-day	18 mg/kg-day
Methemoglobin (% hemoglobin)	0.26 ± 0.11 (N = 13)	0.79 ± 0.15[c] (N = 12)	0.89 ± 0.18[d] (N = 12)	1.97 ± 0.17[d] (N = 13)

[a]NTP, 1989
[b]Mean ± standard error
[c]Statistically significant ($p < 0.05$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.01$) in William's pairwise test versus control

Table A-2. Methemoglobin Levels in Male and Female F344/N Rats Given Gavage Doses of *p*-Chloroaniline for 13 Weeks[a]

Sex	Vehicle Control	5 mg/kg-day	10 mg/kg-day	20 mg/kg-day	40 mg/kg-day	80 mg/kg-day
Males	0.08 ± 0.04	0.59 ± 0.10[d]	0.70 ± 0.24[d]	0.68 ± 0.20[d]	0.68 ± 0.19[d]	0.86 ± 0.16[d]
Females	0.46 ± 0.13	1.35 ± 0.15[c]	1.85 ± 0.18[c]	1.73 ± 0.21[c]	2.40 ± 0.15[c]	3.68 ± 0.45[c]

[a]NTP, 1989
[b]Mean ± standard error (N = 10/sex/group; N = 9 in the male 10 mg/kg-day; N = 9 in the female 80 mg/kg-day)
[c]Statistically significant ($p < 0.05$) in William's pairwise test versus control
[d]Statistically significant ($p < 0.01$) in William's pairwise test versus control

The model fitting procedure for continuous data is as follows. The BMD modeling was conducted with the U.S. EPA's BMD software (BMDS version 1.4.1). For continuous data sets, 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, scaled residue at the range of benchmark response (BMR), and visual inspection of the model fit. Among all the models providing 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 adequate model fit, whether the variance needed to be modeled, and if so, how it was modeled also factors into the determination of final use of the model results. If a homogenous variance model is recommended based on the 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 was negative and the non-

homogenous variance model did not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling.

Methemoglobin. For male rats exposed to *p*-chloroaniline for 6 months, statistical tests indicated that variances were constant across exposure groups (this is reflected in the standard errors listed in Table A-1). The homogeneous variance model adequately fit the variance data for male rats. However, none of the possible continuous models were adequately fitted to the data, as shown by the means' *p*-values (<0.1) in Table A-3, suggesting that the 6 month methemoglobinemia male rat data set (NTP, 1989) is not suitable for BMD modeling.

Table A-3. Model Predictions for Changes in Methemoglobin in Male Rats Exposed to *p*-Chloroaniline by Oral Gavage for 6 Months[a]

Model	BMR	Variance *p*-value[b]	Means *p*-value[c]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Full Data Set Modeled						
Linear (constant variance)	1 SD	0.4121	0.0024	2.9650	3.2989	2.5529
Polynomial[d] (constant variance)	1 SD	0.4121	0.0095	-0.1748	2.2421	1.5685
Power[e] (constant variance)	1 SD	0.4121	0.0095	-0.1748	2.2421	1.5685
Hill[f] (constant variance)	1 SD	0.4121	0.0023	1.8309	2.2375	NA[g]
Highest Dose Dropped						
Linear (constant variance)	1 SD	0.3144	0.0765	-5.6664	5.4469	3.3615
Polynomial[d] (constant variance)	1 SD	0.3144	0.0765	-5.6664	5.4469	3.3615
Power[e] (constant variance)	1 SD	0.3144	0.0765	-5.6664	5.4469	3.3615

[a]NTP, 1989
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria
[d]1-Degree polynomial
[e]Power restricted to ≥ 1
[f]N restricted to >1
[g]NA = Not available (model failed)

For male rats exposed for 13 weeks, the variances were not constant across exposure groups (this is reflected in the variance p-values in Table A-2). For the majority of modeling outputs, the non-homogeneous variance model adequately fit the variance data for males. However, an adequate fit to the means data could not be achieved by any model, even when the 3 highest-dose groups were excluded, leaving 3 dose groups for model fitting (Table A-4). For females, under a condition of homogenous variance, the linear, polynomial, and power models provided equivalent adequate fit to the data when the 3 highest dose groups were excluded (Table A-5). A plot of the resulting linear model of the truncated female data is shown in Figure A-2, which can be considered representative of all three fitted models. The resulting BMD and BMDL were 3.39 and 2.55 mg/kg-day, respectively.

Table A-4. Model Predictions for Changes in Methemoglobin in Male Rats Exposed to *p*-Chloroaniline by Oral Gavage for 13 Weeks[a]

Model	BMR	Variance *p*-value[b]	Means *p*-value[c]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Full Data Set Modeled						
Linear (constant variance)	1 SD	<0.0001	0.1068	-11.86	89.6169	52.4222
Linear (modeled variance)	1 SD	0.3331	<0.0001	-7.8814	86.5447	41.0661
Polynomial[d] (modeled variance)	1 SD	0.3331	<0.0001	-7.8814	86.5448	41.0661
Power[e] (modeled variance)	1 SD	0.3331	<0.0001	-5.8814	86.5448	41.0661
Hill[f] (modeled variance)	NA[g]					
Highest Dose Dropped						
Linear (constant variance)	1 SD	<0.001	0.0825	-7.5792	55.1238	28.6857
Linear (modeled variance)	1 SD	0.3474	<0.0001	-9.5967	3.8382	1.9897
Polynomial[h] (modeled variance)	1 SD	0.3474	<0.0001	-9.5967	3.8382	1.9897
Power[e] (modeled variance)	1 SD	0.3474	<0.0001	-11.6807	5.1868	3.2291
Hill[f] (modeled variance)	1 SD	0.3474	0.4632	-32.6000	4.4602	NA[g]
2 Highest Doses Dropped						
Linear (constant variance)	1 SD	<0.0001	0.1102	-8.5420	20.2096	11.6515
Linear (modeled variance)	1 SD	0.1919	0.0103	-26.3871	1.9728	1.1984
Polynomial[i] (modeled variance)	1 SD	0.1919	0.0025	-26.3871	1.9728	1.1984
Power[e] (modeled variance)	1 SD	0.1919	0.0025	-24.3871	1.9728	1.1984
Hill[f] (modeled variance)	NA[g]					
3 Highest Doses Dropped						
Linear (constant variance)	1 SD	<0.0001	0.2600	-12.1653	7.4723	4.6989
Linear (modeled variance)	1 SD	0.0708	0.8703	-34.9265	1.3314	0.8335
Polynomial[j] (modeled variance)	1 SD	<0.0001	0.7476	-32.7029	1.5817	0.9864
Power[e] (modeled variance)	1 SD	<0.0001	0.7476	-32.7029	1.5817	0.9864

[a]NTP, 1989
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria
[d]4-Degree polynomial
[e]Power restricted to ≥ 1
[f]N restricted to >1
[g]NA = Not available (model failed)
[h]3-Degree polynomial
[i]2-Degree polynomial
[j]1-Degree polynomial

Table A-5. Model Predictions for Changes in Methemoglobin in Female Rats Exposed to *p*-Chloroaniline by Oral Gavage for 13 Weeks[a]

Model	BMR	Variance *p*-value[c]	Means *p*-value[d]	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)
Full Data Set Modeled						
Linear (constant variance)	1 SD	<0.0001	0.0329	33.9254	22.8570	18.5298
Linear (modeled variance)	1 SD	0.0722	0.0008	28.5505	14.6935	10.433
Highest Dose Dropped						
Linear (constant variance)	1 SD	0.5329	0.0004	2.5959	15.3352	11.774
Polynomial[d] (constant variance)	1 SD	0.5329	<0.0001	2.5959	15.3352	11.774
Power[e] (constant variance)	1 SD	0.5329	<0.0001	6.5959	15.3352	11.774
Hill[f] (constant variance)	1 SD	0.5329	0.0261	6.8953	2.3202	1.1613
2 Highest Doses Dropped						
Linear (constant variance)	1 SD	0.3849	0.0007	3.9938	10.4566	7.4041
Polynomial[g] (constant variance)	1 SD	0.3849	0.0001	3.9938	10.4566	7.4041
Power[e] (constant variance)	1 SD	0.3849	0.0001	7.9938	10.4566	7.4041
Hill[f] (constant variance)	NA[h]					
3 Highest Doses Dropped						
Linear (constant variance)	1 SD	0.5752	0.2813	-9.0687	3.3944	2.5505
Polynomial[i] (constant variance)	1 SD	0.5752	0.2813	-9.0687	3.3944	2.5505
Power[e] (constant variance)	1 SD	0.5752	0.2813	-9.0687	3.3944	2.5505

[a]NTP, 1989
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria[c] 3-Degree polynomial
[d]Power restricted to ≥ 1
[e]N restricted to >1
[f]2-Degree polynomial
[g]NA = Not available (model failed)
[h]1-Degree polynomial

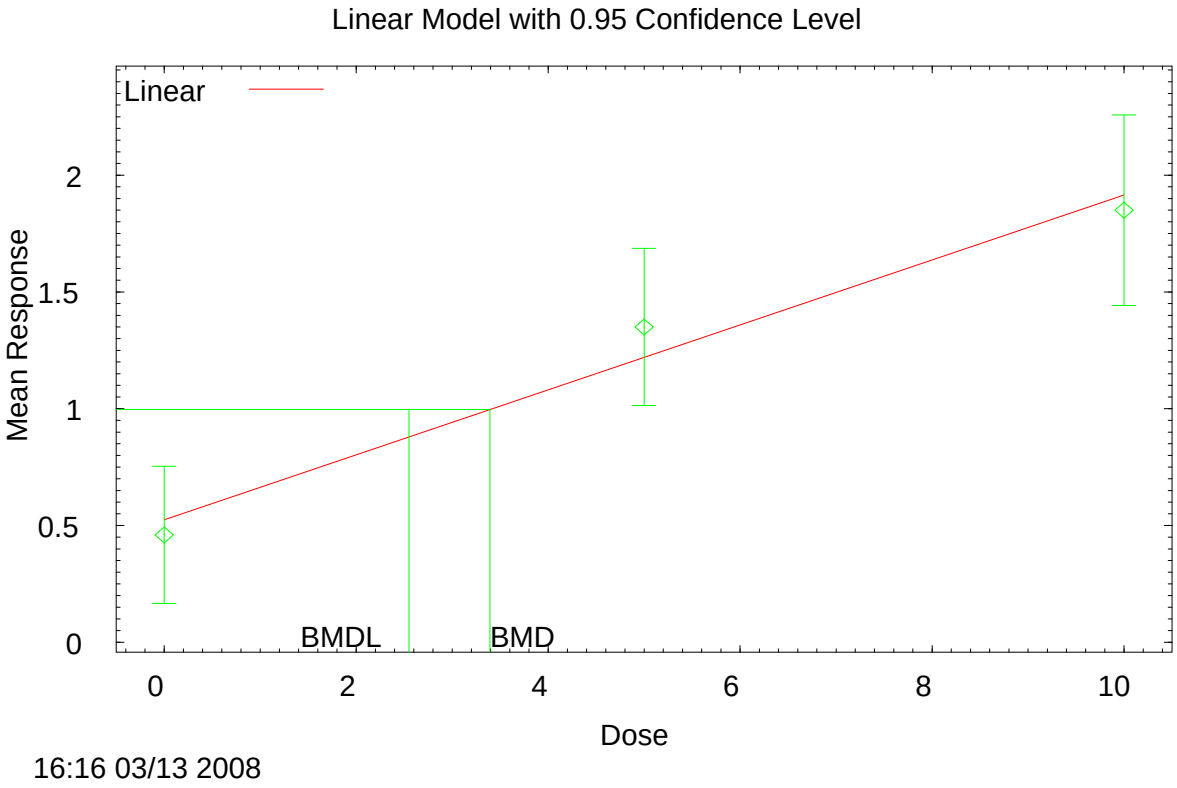

Figure A-2. Benchmark Dose Modeling (Linear Model) Results for Changes in Methemoglobin in Female Rats Exposed to *p*-Chloroaniline by Oral Gavage for 13 Weeks by NTP (1989)

APPENDIX B. DETAILS OF BMD ANALYSIS FOR THE CANCER ORAL SLOPE FACTOR FOR *p*-CHLOROANILINE

Quantal Data

The multistage-cancer model in the U.S. EPA BMDS (version 1.4.1) software program was fit to the incidence data for splenic tumors (fibroma, osteosarcoma, or hemangioma) in male rats (NCI, 1979; NTP, 1989) (see Tables 5 and 7), adrenal tumors (pheochromocytoma or malignant pheochromocytoma) in male rats (NTP, 1989) (see Table 8), and hepatocellular carcinoma in male mice (NTP, 1989) (see Table 9). Predicted doses (BMDs and BMDLs) associated with 10% extra risks were calculated.

Multistage-cancer modeling outputs from the BMDS program (Tables B-1 to B-4) were evaluated using the criteria described in U.S. EPA (2000). Goodness-of-fit was evaluated using the chi-square statistic calculated by the BMDS program. Local fit is evaluated visually on the graphic output (see Figures B-1 to B-4) by comparing the observed and estimated results at each data point. Acceptable global goodness-of-fit is indicated by a *p*-value greater than or equal to 0.1. The multistage-cancer model met these criteria for all tumor datasets modeled, except for combined incidence of liver adenoma or carcinoma (see Table B-5). Since the multistage-cancer model did not provide an adequate fit for combined liver tumor incidence, all dichotomous models in the U.S. EPA BMDS (version 1.4.1) software program were attempted in order to identify an acceptable global goodness-of-fit. As seen in Table B-5, all but the log-logistic model failed; the log-logistic fit barely met the criteria (i.e., *p*-value = 0.1007). This BMD modeling result suggests that the combined incidence of liver adenoma or carcinoma may not be the most optimal dose-response data on which to base a subsequent OSF derivation. This may be partly due to the observed negative trend in liver adenoma incidence with increasing dose (i.e. adenomas may be transitioning to carcinomas). As such, liver carcinomas only were further considered (see Table B-4).

Table B-1. Goodness-of-Fit Statistics and BMD_{10}s and $BMDL_{10}$s from Multistage-Cancer Model Fit to Incidence Data for Splenic Tumors (Fibroma, Fibrosarcoma, Hemangiosarcoma, Osteosarcoma, or Sarcoma NOS) in Male Rats Exposed to *p*-Chloroaniline by Diet for 78 Weeks[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage[b,c]	2	2.83	0.2429	56.518	19.221	15.084

[a]NCI, 1979
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]2-degree polynomial; lowest degree polynomial with adequate fit

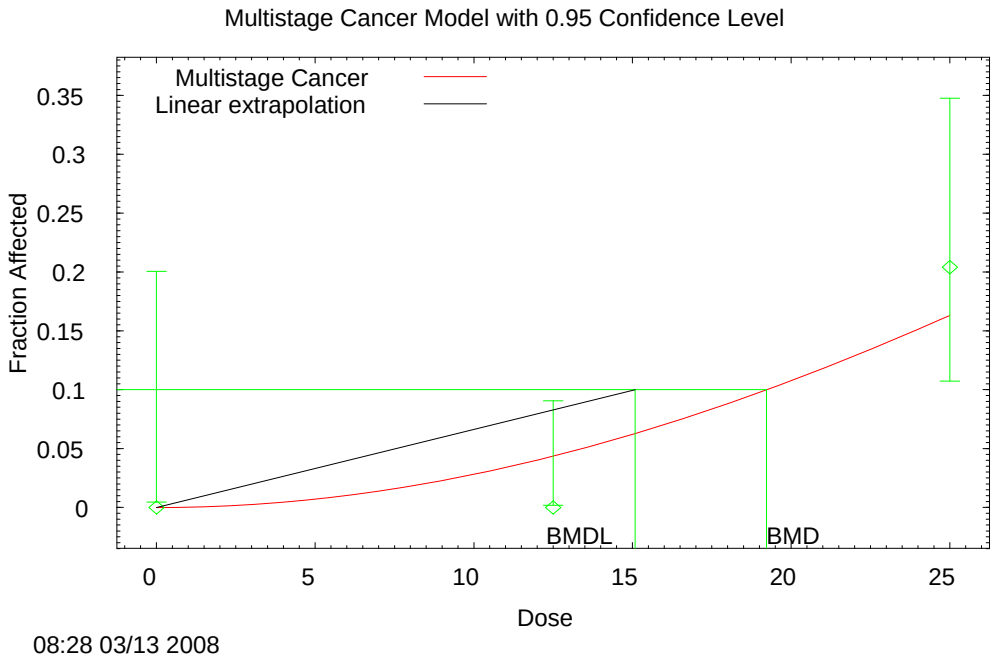

Figure B-1. Observed and Predicted Incidences of Splenic Tumors (Fibroma, Fibrosarcoma, Hemangiosarcoma, Osteosarcoma, or Sarcoma NOS) in Male Rats Exposed to *p*-Chloroaniline by Diet for 78 Weeks by NCI (1979)

Table B-2. Goodness-of-Fit Statistics and BMD_{10}s and $BMDL_{10}$s from Multistage-Cancer Model Fit to Incidence Data for Splenic Tumors (Fibrosarcoma, Osteosarcoma, or Hemangioma) in Male Rats Exposed to *p*-Chloroaniline by Gavage for 104 Weeks[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage[c]	3	2.72	0.4367	92.829	5.1852	4.2398

[a]NTP, 1989
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]2-degree polynomial; lowest degree polynomial with adequate fit

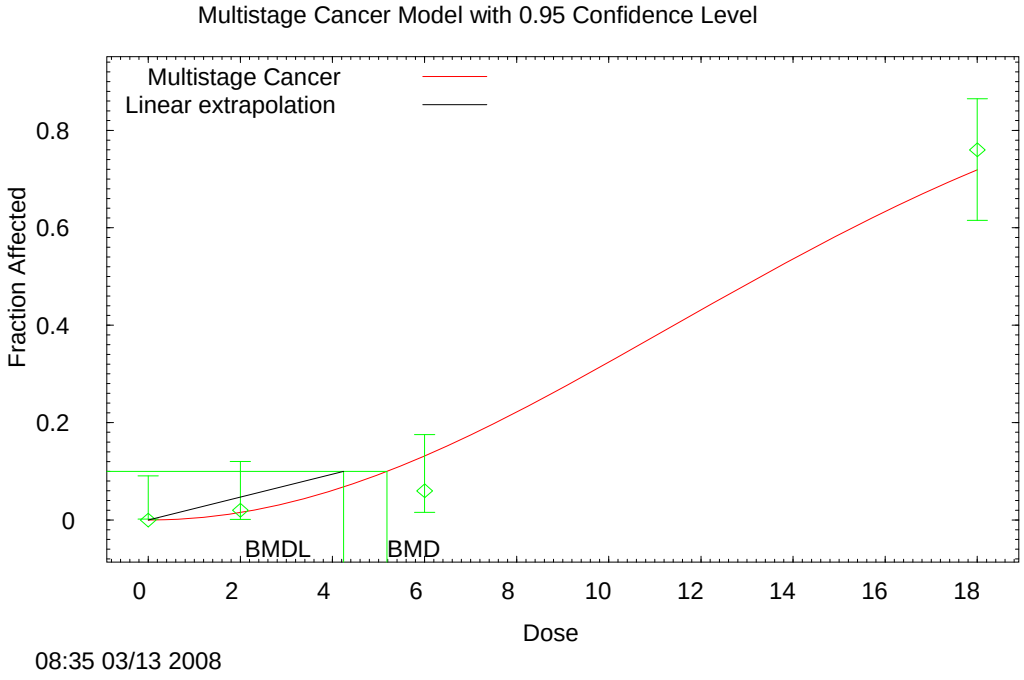

Figure B-2. Observed and Predicted Incidences of Splenic Tumors (Fibrosarcoma, Osteosarcoma or Hemangioma) in Male Rats Exposed to *p*-Chloroaniline by Gavage for 104 Weeks by NTP (1989)

Table B-3. Goodness-of-Fit Statistics and BMD_{10}s and $BMDL_{10}$s from Multistage-Cancer Model Fit to Incidence Data for Adrenal Tumors (Pheochromocytoma or Malignant Pheochromocytoma) in Male Rats Exposed to *p*-Chloroaniline by Gavage for 104 Weeks[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage[c]	2	0.44	0.8042	246.456	4.4067	2.6700

[a]NTP, 1989
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]1-degree polynomial; lowest degree polynomial with adequate fit

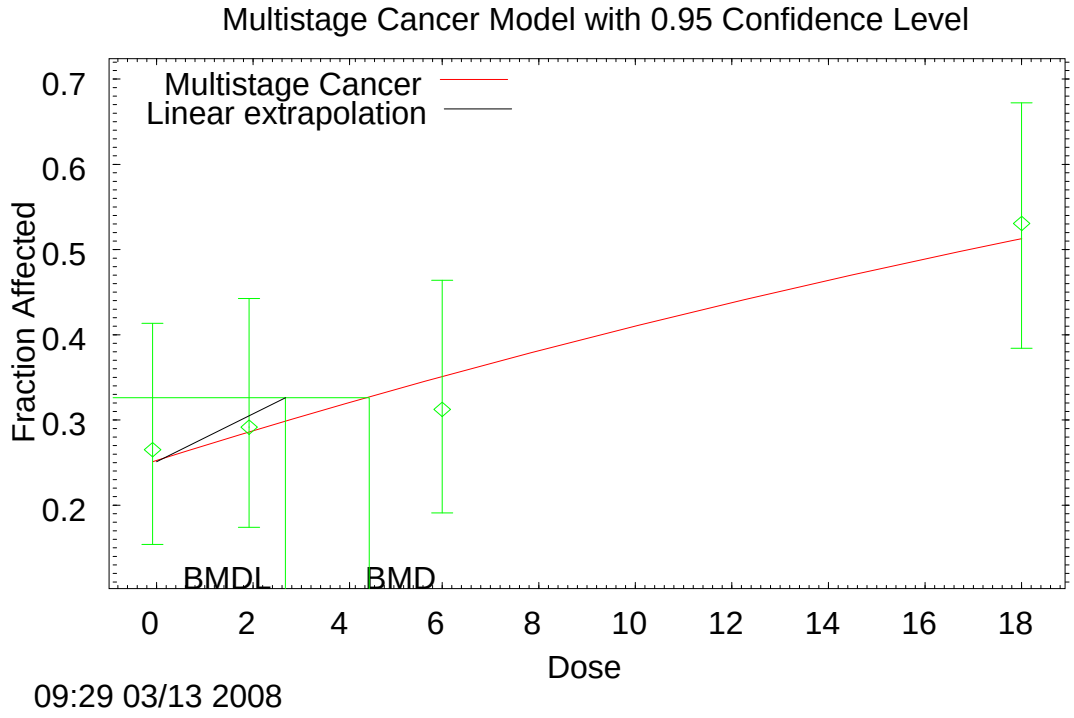

Figure B-3. Observed and Predicted Incidences of Adrenal Tumors (Pheochromocytoma or Malignant Pheochromocytoma) in Male Rats Exposed to *p*-Chloroaniline by Gavage for 104 Weeks by NTP (1989)

Table B-4. Goodness-of-Fit Statistics and BMD_{10}s and $BMDL_{10}$s from Multistage-Cancer Model Fit to Incidence Data for Hepatocellular Carcinoma in Male Mice Exposed to *p*-Chloroaniline by Gavage for 104 Weeks[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage[c]	2	1.13	0.5694	184.825	8.5835	5.5352

[a]NTP, 1989
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]1-degree polynomial; lowest degree polynomial with adequate fit

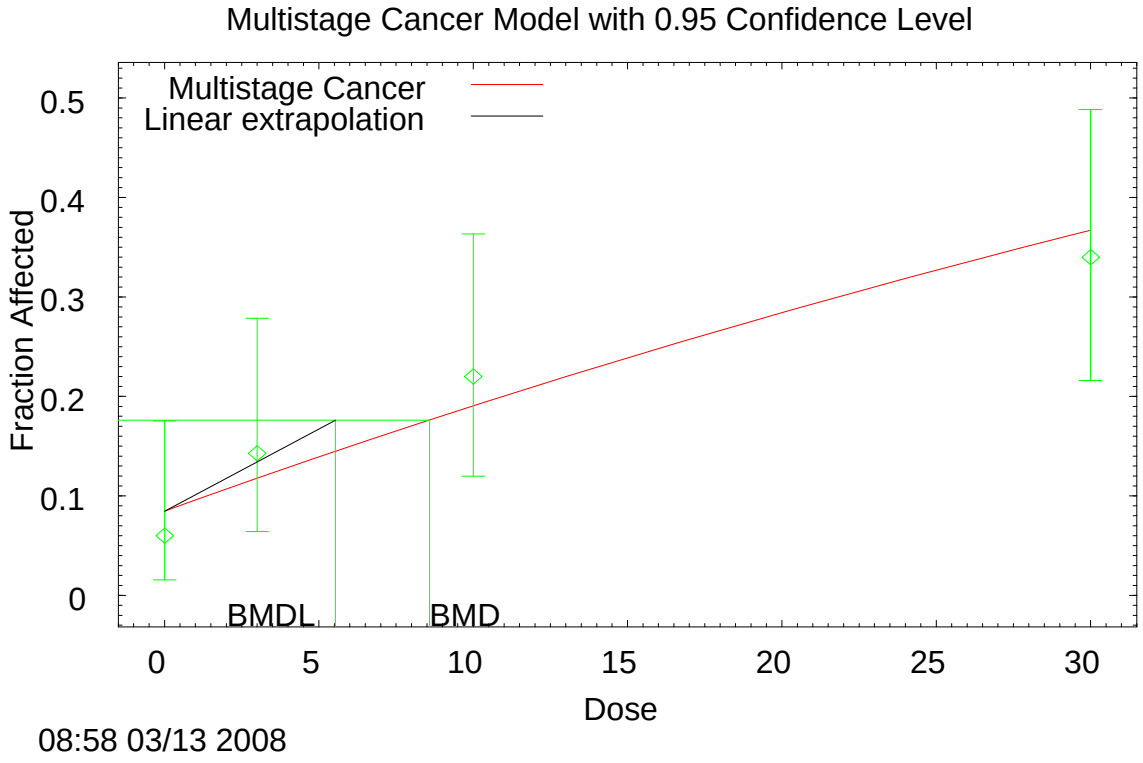

Figure B-4. Observed and Predicted Incidences of Hepatocellular Carcinoma in Male Mice Exposed to *p*-Chloroaniline by Gavage for 104 Weeks by NTP (1989)

Table B-5. Goodness-of-Fit Statistics and BMD_{10}s and $BMDL_{10}$s from Model Fits to Incidence Data for Hepatocellular Adenoma or Carcinoma in Male Mice Exposed to *p*-Chloroaniline by Gavage for 104 Weeks[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Gamma[c]	2	4.68	0.0964	263.708	15.3749	6.5547
Logistic	2	4.79	0.0911	263.851	17.9038	8.9416
Log-logistic[d]	2	4.59	0.1007	263.601	13.5748	5.0319
Multistage[e]	2	4.68	0.0964	263.708	15.3749	6.5547
Probit	2	4.78	0.0914	263.840	17.7150	8.7761
Log-probit[d]	2	5.47	0.0650	264.613	25.6893	12.7162
Quantal linear	2	4.68	0.0964	263.708	15.3749	6.5547
Weibull[c]	2	4.68	0.0964	263.708	15.3749	6.5547

[a]NTP, 1989
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]Power restricted to ≥1
[d]Slope restricted to ≥1
[e]Betas restricted to ≥0