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Provisional Peer Reviewed Toxicity Values for

Aniline
(CASRN 62-53-3)

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

Acronyms and Abbreviations

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

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

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR ANILINE (CASRN 62-53-3)

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.

This document has passed the STSC quality review and peer review evaluation indicating that the quality is consistent with the SOPs and standards of the STSC and is suitable for use by registered users of the PPRTV system.

INTRODUCTION

An RfD for aniline is not available on IRIS (U.S. EPA, 2007), the HEAST (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). The CARA list (1991, 1994) includes a Health and Environmental Effects Profile (HEEP) for Aniline (U.S. EPA, 1985) that did not calculate an RfD, presumably because chronic oral carcinogenicity data were available to calculate a slope factor. A draft Health and Environmental Effects Document prepared for EPA by Syracuse Research Corporation (U.S. EPA, 1992) derived an oral RfD for aniline of 7E-3 mg/kg-day based on a LOAEL of 10 mg/kg-day for erythrocytic and splenic toxicity in rats exposed to aniline hydrochloride in the diet for 104 weeks (CIIT, 1982). The LOAEL was converted to the equivalent of aniline (7 mg/kg-day) and divided by an uncertainty factor of 1000 (10 for the use of a LOAEL, 10 to account for extrapolation from animal data and 10 to protect sensitive individuals) to derive the RfD. Aniline has not been the subject of a toxicological profile by ATSDR (2006) or the WHO (2006).

IRIS (U.S. EPA, 2007) lists a chronic RfC of 1E-3 mg/m^3 for aniline, based on a NOAEL of 19 mg/m^3 in an inhalation study in rats, mice, and guinea pigs exposed to aniline for 6 hours/day, 5 days/week for 20-26 weeks (Oberst et al., 1956) and a LOAEL of 64.7 mg/m^3 for mild spleen toxicity in rats exposed for 6 hours/day, 5 days/week for 2 weeks (DuPont deNemours, 1982).

IRIS (U.S. EPA, 2007) has classified aniline as a probable human carcinogen (Group B2), based on an increased incidence of sarcomas of the spleen and other organs in two strains of rats exposed orally to aniline hydrochloride (CIIT,1982; NCI, 1978) and supporting genetic toxicity evidence. An oral slope factor of 5.7E-3/mg/kg/day and a drinking water unit risk of 1.6E-7/µg/L are available on IRIS (U.S. EPA, 2007). An inhalation unit risk for aniline is not available on IRIS (U.S. EPA, 2007) or the HEAST (U.S. EPA, 1997). The HEEP for Aniline (U.S. EPA, 1985) found no information regarding carcinogenicity in humans or animals exposed to aniline by inhalation; the oral cancer assessment in this document was based on the NCI (1978) rat oral bioassay. The draft HEED on Aniline (U.S. EPA, 1992) reported that no data were available on the carcinogenicity of inhaled aniline, but derived an inhalation unit risk of 1.6E-6 per µg/m^3 for aniline by extrapolation from the CIIT (1982) oral data on aniline hydrochloride that were used for deriving the oral slope factor on IRIS (U.S. EPA, 2007). The ACGIH (1992, 2006) evaluation for aniline includes an A3 notation for "confirmed animal carcinogen with unknown relevance to humans," based on the NCI (1978) oral rat cancer bioassays, but includes no inhalation carcinogenicity data. IARC (1974, 1982, 1987) found no data regarding carcinogenicity of inhaled aniline and concluded that aniline is not classifiable as to its carcinogenicity to humans (Group 3). The inhalation carcinogenicity of aniline has not been investigated by the NTP.

Computer searches for information on the toxicity of aniline were conducted for the period from 1990-1996 in Toxline, RTECS, TSCATS, and DART. Update literature searches were conducted from 1995 to August 2001 in TOXLINE, CANCERLIT (1984-2001), MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. Monographs by IARC (1974, 1982) and a toxicity review of aromatic nitroso and amino compounds (Weisburger and Hudson, 2001) were consulted for relevant information for aniline. An additional literature search was conducted by NCEA-Cincinnati using TOXLINE, MEDLINE and Chemical and Biological Abstracts databases for the period August 2001 to November 3, 2006.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure. Quantitative data on the oral toxicity of aniline in humans are limited to an acute exposure study conducted by Jenkins et al. (1972). In this study, 20 (17 male and 3 female) subjects received 5, 15 or 25 mg oral doses of aniline administered on three consecutive days. As an extension of this study, some of the same volunteers later received aniline doses of 35 mg (5 subjects), 45 mg (5 subjects), 55 mg (2 subjects) or 65 mg (1 subject); the authors did not specify whether these were administered as single doses or on three days. Urine and blood

samples from each subject were analyzed before treatment; urine was evaluated for glucose and protein, and blood was examined for hemocytology and evidence of a deficiency of erythrocyte glucose-6-phosphate dehydrogenase. Urine samples obtained six hours after the last dose of aniline were tested for urobilinogen, glucose and protein. Blood obtained from a pricked finger 1, 2, 3 or sometimes 4 hours after treatment was evaluated for methemoglobin. In addition, blood obtained by venipuncture 24 hours after each dose of aniline was evaluated for methemoglobin, Heinz bodies, packed cell volume, hemocytology, and blood chemistry. The mean of the maximum detected methemoglobin level in the 1- to 3-hour fingerprick blood samples for each group was compared to the levels in the 5-mg group to determine if aniline exposure resulted in significant alterations. No significant increase in methemoglobin levels was detected 1-3 hours after administration of 5 or 15 mg. Significantly higher methemoglobin levels were observed at doses at or above 25 mg. The highest observed increase was observed in one subject two hours after administration of 65 mg, but the level was within normal limits one hour later. Thus, the NOAEL and LOAEL for this study are 15 and 25 mg, respectively (equivalent to 0.21 and 0.36 mg/kg doses, respectively, assuming a reference body weight of 70 kg). No additional human oral data were located in the review documents (U.S. EPA, 1985, 1992; IARC, 1974, 1982; Weisburger and Hudson, 2001) or the literature search.

Inhalation Exposure. The availability of a chronic RfC for aniline on IRIS (EPA, 2007) precludes review of pertinent noncancer toxicity data for inhalation exposure. No studies were located in review documents (U.S. EPA, 1985, 1992; IARC, 1974, 1982; Weisburger and Hudson, 2001) or in the literature search regarding carcinogenicity of inhaled aniline to humans.

Animal Studies

Oral Exposure. The general outline of toxicity in rats orally exposed to aniline hydrochloride primarily involves increased methemoglobin formation in erythrocytes, leading to decreases in hemoglobin, hematocrit, and/or erythrocyte levels (CIIT, 1982; NCI, 1978; Khan et al., 1993; CIIT, 1977). Compensatory increases in hematopoiesis, including reticulocyte levels, are observed in response to these anemic effects. Splenic toxicity (hemosiderin deposition, extramedullary hematopoiesis, capsulitis and congestion) is caused by the accumulation of large numbers of damaged erythrocytes. Mice appear to be less vulnerable than rats to the toxic effects of aniline (NCI, 1978), which could be related to the higher activity of erythrocyte methemoglobin reductase in that species (Smith, 1995). A discussion of the chronic (CIIT, 1982; NCI, 1978) and subchronic (Khan et al., 1993; CIIT, 1977) oral toxicity studies follows.

In a study conducted for CIIT (1982), groups of 130 male and 130 female CD-1 rats were fed diets containing aniline hydrochloride at target doses of 0, 10, 30 or 100 mg/kg-day for 104 weeks. Actual doses were within 5% of the target doses, as determined by analysis of aniline hydrochloride concentrations in feed samples and measurement of food intake and body weight. Groups of 10 rats per sex were killed after 26 and 52 weeks, groups of 20 rats per sex were killed after 78 weeks of exposure and the remainder at 104 weeks. Animals were examined twice daily for mortality and clinical signs. Food consumption and body weights were recorded weekly for the first fourteen weeks, biweekly for the next twelve weeks and every fourth week thereafter. Hematology, clinical chemistry and urinalysis parameters were measured in ten rats per sex per group at 26 and 52 weeks and in twenty rats per sex per group at 78 and 104 weeks. All rats

were given an ophthalmoscopic examination in the week prior to scheduled sacrifice. Gross necropsies were conducted on all rats sacrificed on schedule, sacrificed *in extremis* or dying early during the study. Organ weights were determined for the brain, heart, liver, kidneys and testes with epididymides at all four timepoints, for the ovaries beginning at week 52, and for the spleen beginning at week 78. For each rat, more than 30 tissues were preserved; histopathological examination was conducted on all tissues from the control and high-dose groups, and the spleen and tissues with gross lesions in the low- and mid-dose groups. Histopathology results were not presented for the entire group of control male and female rats sacrificed at 104 weeks; results for 47/sex were provided for controls (compared to 64-77/sex for treated groups), but no explanation was given for the omissions. Most of the males omitted comprised the subset of 20 that had been exsanguinated for hematology and clinical chemistry data.

The survival rate was significantly lower in the 100 mg/kg-day male rats, but was unaffected in treated females. A "hunched appearance" was more frequently observed in the rats exposed to aniline hydrochloride than in the controls. The incidence and severity of ophthalmoscopic findings (cataracts) increased with dose. Food consumption and body weight gain were not adversely affected in treated rats compared to controls. Significant treatment-related clinical chemistry elevations were observed only at 26 weeks: in alkaline phosphatase levels in both sexes at ≥30 mg/kg-day and in blood urea nitrogen in males at 100 mg/kg-day and in females at ≥10 mg/kg-day. No aniline-related alterations in urinalysis results were observed.

Hematological examinations demonstrated dose-related toxicity to erythrocytes in both sexes, although the changes were not always statistically different from the controls (Table 1). Statistically significant changes include the following. After 26 weeks of treatment, the blood methemoglobin concentration was increased in males at ≥30 mg/kg-day and females at 100 mg/kg-day. Erythrocyte counts were reduced in high-dose males and mid- and high-dose females. The number of erythrocytes with Heinz bodies was increased in high-dose males but not females. In females, hemoglobin concentration was reduced at ≥30 mg/kg-day and hematocrit was reduced at 100 mg/kg-day, although these parameters were not significantly affected in males. In both sexes at ≥30 mg/kg-day, reticulocyte counts were increased, indicating regenerative erythropoiesis subsequent to hemolytic anemia. After 52 weeks, methemoglobin levels were significantly elevated only in females at ≥30 mg/kg-day, but Heinz bodies were observed in both sexes at 100 mg/kg-day. At ≥10 mg/kg-day, erythrocyte counts were reduced in both sexes, and hemoglobin and hematocrit were reduced in males; hemoglobin was reduced in females at ≥30 mg/kg-day. Reticulocyte counts were elevated in males at ≥30 mg/kg-day and females at 100 mg/kg-day. Hematology values at 78 weeks were similar to the 52 week values. Methemoglobin levels were elevated in males at ≥30 mg/kg-day, but Heinz bodies were observed in both sexes at the high dose. Dose-related reductions in hemoglobin, hematocrit (males only), and erythrocyte counts were observed in males at ≥10 mg/kg-day and females at ≥30 mg/kg-day. Reticulocyte counts were elevated in mid- and high-dose males and all treated female groups. At 104 weeks, no hematological effects were observed in the 10-mg/kg-day groups. Methemoglobin was elevated only in high-dose males and Heinz bodies

Table 1. Statistically Significant Changes* in Selected Hematological Parameters Observed in Rats Exposed to Aniline Hydrochloride in the Diet for 26-104 Weeks (CIIT, 1982)

	26 Weeks				52 Weeks				78 Weeks				104 Weeks			
Dose (mg/kg-day)	0	10	30	100	0	10	30	100	0	10	30	100	0	10	30	100
Males																
Hematocrit	48.05	46.65	45.6	45.5	47.2	45.2 (-4.2)	45.1 (-4.4)	42.6 (-9.7)	54.55	46.4 (-14.9)	44.45 (-18.5)	41.85 (-23.3)	57.8	55.67	50.25 (-13.1)	43.5 (-24.7)
Hemoglobin	17.49	17.04	16.7	16.19	16.17	14.98 (-7.4)	15.02 (-7.1)	13.67 (-15.5)	18.91	16.24 (-14.1)	14.85 (-21.5)	13.4 (-29.1)	19.46	18.19	15.67 (-19.5)	13.56 (-30.3)
Erythrocyte	9.173	8.746	8.406	7.817 (-14.8)	9.127	8.527 (-6.6)	8.504 (-6.8)	7.538 (-17.4)	9.6	8.459 (-11.9)	7.735 (-19.4)	7.209 (-24.9)	9.262	8.844	7.552 (-18.5)	7.552 (-18.5)
Heinz bodies**	0	0	0.01	2.78#	0	0	0	1.67#	0	0	0.01	1.21#	0	0	0	0
Reticulocyte	1.66	2.07	2.44 (+47)	4.35 (+162)	0.46	0.63	1.52 (+230)	3.09 (+572)	1.99	2.42	3.35 (+68)	4.47 (+125)	3.08	3.10	3.84	7.47 (+143)
Methemoglobin	1.08	1.48	2.49 (+131)	2.55 (+136)	1.87	2.67	3.03	2.42	0.96	1.53	2.51 (+161)	2.35 (+145)	1.39	1.89	1.4	3.63 (+161)
Females																
Hematocrit	49.25	49.05	47.15	45.1 (-8.4)	46.8	46.35	45.85	44.25	46.67	46.4	44.7	43.92 (-5.9)	45.82	45.47	45.02	42.57 (-7.1)
Hemoglobin	16.89	16.46	15.57 (-7.8)	15.04 (-11.0)	15.67	14.42	13.56 (-13.5)	13.53 (-13.7)	16.34	15.76	14.79 (-9.5)	14.27 (-12.7)	15.08	15.29	15.26	13.97 (-7.4)
Erythrocyte	9.033	8.519	7.965 (-11.8)	7.406 (-18.0)	8.414	7.519 (-10.6)	7.280 (-13.5)	6.402 (-23.9)	8.322	8.271	7.452 (-10.5)	7.046 (-15.5)	8.199	7.999	7.856	7.137 (-13.0)
Heinz bodies**	0	0	0	0.17	0	0	0	0.56#	0.02	0.02	0.03	0.21#	0	0	0	0
Reticulocyte	0.81	1.10	1.73 (+114)	3.79 (+368)	1.69	1.85	2.03	4.57 (+170)	1.80	2.52 (+40)	3.64 (+102)	5.05 (+180)	1.25	1.15	2.22 (+78)	4.29 (+243)
Methemoglobin	2.06	2.45	2.36	2.95 (+13.5)	1.12	1.16	2.05 (+83)	1.64 (+46.4)	1.95	1.13	1.99	2.37	2.72	3.23	3.38	3.03

*In each data cell, the first row shows the group mean value; the second row shows statistically significant changes (% difference from control) in parentheses.
**For Heinz bodies, statistically significant increases are marked with a pound sign (#).
Units: Hematocrit (%); Hemoglobin (g/dL); Erythrocytes (x 10^6/mm^3); Heinz Bodies (%); Reticulocytes (%); Methemoglobin (%)

were not detected in either sex. Evidence of erythrocyte destruction – reductions in erythrocyte count, hemoglobin concentration and hematocrit – were observed in males at ≥30 mg/kg-day and females at 100 mg/kg-day. Reticulocyte counts were elevated in high-dose males and mid- and high-dose females.

At 52, 78 and 104 weeks, statistically significant alterations in lung, liver, kidney, spleen and/or ovary weights were observed in male or female rats. Spleen weight increases in males were only observed at 78 weeks: absolute weight at ≥30 mg/kg-day and relative weight at 100 mg/kg-day. In females, absolute and relative spleen weights increased at ≥30 mg/kg-day at 78 weeks and at 100 mg/kg-day at 104 weeks. In males at 104 weeks, absolute lung weights were lower in the 10 and 100 mg/kg-day groups and relative lung weights were lower in the 10 and 30 mg/kg-day groups. Relative liver weights were increased in high-dose males at 52 weeks and in high-dose females at 78 and 104 weeks. Relative kidney weights were increased in the 100 mg/kg-day females at 78 weeks. Absolute and relative ovary weights were decreased in 100 mg/kg-day females at 104 weeks. Other sporadic changes in absolute or relative organ weights also occurred at all doses beginning at 52 weeks.

Consistent with the organ weight observations, the spleen demonstrated aniline-related histopathological changes at lower doses than other target organs; incidence and severity data are presented for male rats in Table 2 and for female rats in Table 3. Since one of the functions of the spleen is to remove damaged erythrocytes from the circulation, splenic deposition of hemosiderin pigment, an iron-containing breakdown product of hemoglobin, was observed in all groups. However, after 52 weeks of treatment, the severity of splenic hemosiderin deposition was generally higher in the treated groups compared to the controls. Approximating the observed increases in reticulocyte counts, the intensity of extramedullary hematopoiesis in the splenic red pulp was increased in the male and female rats exposed at ≥30 mg/kg-day for 26 weeks and in those exposed at ≥10 mg/kg-day for 52, 78 or 104 weeks. Capsulitis of the spleen was not observed in any of the control rats, but occurred in all treated groups. In both sexes at 26 weeks, capsulitis was focal or multifocal in the 10 and 30 mg/kg-day groups, but chronic in the 100 mg/kg-day groups. At 52, 78 and 104 weeks, chronic capsulitis was largely restricted to the 100 mg/kg-day groups, although a few rats in the 30 mg/kg-day groups were affected. In the male rats, the severity of capsulitis progressed from minimal-to-moderate to moderate-to-severe; the severity of capsulitis was lower in the female rats. Congestion was observed in the spleens of male rats exposed at 100 mg/kg-day 52 weeks, or exposed at ≥10 mg/kg-day for 78 or 104 weeks; in females, splenic congestion was observed following exposure at ≥30 mg/kg-day for 78 weeks or 100 mg/kg-day for 104 weeks. Congestion ranged from moderate to severe in the majority of high-dose rats. The following splenic lesions were also observed (not listed in Tables 3 and 4). Moderate-to-severe stromal hyperplasia was observed in the 100 mg/kg-day groups at 78 and 104 weeks; the incidence at 104 weeks was approximately 48% for males and

Table 2. Incidence and Severity of Splenic Nonneoplastic Lesions in Male Rats Exposed to Aniline Hydrochloride in the Diet for 26-104 Weeks (CIIT, 1982)

	26 Weeks				52 Weeks				78 Weeks				104 Weeks			
Dose (mg/kg-day) (N)	0 (10)	10 (10)	30 (10)	100 (10)	0 (10)	10 (10)	30 (10)	100 (10)	0 (20)	10 (20)	30 (20)	100 (20)	0 (47)	10 (75)	30 (74)	100 (64)
Finding Grade																
Hemosiderin 0	2									1		2	3	2		3
1	2	7	6	1	4			4	7	2	1	1	17	20	2	1
2	6	3	3	9	6	6	3	6	10	9	4	6	18	42	33	16
3			1			4	7		3	8	15	11	9	9	34	39
4														2	5	5
Extramedullary 0	5	5	1		2				3				5	1		3
Hematopoiesis 1	4	5			8	1			11				5	6		2
2	1		8	5		9	10	9	6	6	1		28	38	13	12
3			1	5				1		13	17	18	9	29	51	32
4										1	2	2		2	10	11
5																3
Congestion 0	10	10	10	10	10	10	10	1	20				46	46	5	13
1														4	1	
2										1			2	23	34	
3										19		1		2	33	15
4								9			20	3			1	36
5												16				
Capsulitis 0	10	3	2		10	4	2		20	20	14		47	73	69	3
1		5	4			6	3				6			1	1	
2		2	4				5								2	6
3				5				3				7		1	2	30
4				4				7				10				25
5				1								3				

Grade: 0 = not observed; 1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe.

Table 3. Incidence and Severity of Splenic Nonneoplastic Lesions in Female Rats Exposed to Aniline Hydrochloride in the Diet for 26-104 Weeks (CIIT, 1982)

	26 Weeks				52 Weeks				78 Weeks				104 Weeks			
Dose (mg/kg-day) (N)	0 (9)	10 (10)	30 (10)	100 (10)	0 (10)	10 (10)	30 (10)	100 (10)	0 (20)	10 (20)	30 (20)	100 (20)	0 (47)	10 (69)	30 (76)	100 (77)
Finding Grade																
Hemosiderin 0		1												1		1
1	1	9	1										1	4		2
2	2		9	1	8	2		1	14			4	21	20	11	25
3	6			9	2	8	10	9	6	13	10	16	25	44	62	47
4										7	9				2	
5											1					
Extramedullary 0	2	3											1	2		2
Hematopoiesis 1	3	5	2						4				5	1		1
2	3	2	8	8	9	6	1	2	13	3		12	30	24	19	8
3	1			2	1	4	9	8	3	13	12	8	10	39	53	62
4										4	8		1	3	4	4
Congestion 0	9	10	10	10	10	10	10	10	20	17	3	2	45	69	72	2
1										3						
2											5				2	2
3											12	6	2		2	63
4												12				10
Capsulitis 0	9	5	6	2	10	10	6		20	20	17	2	47	66	72	7
1		2	1	1			3	3			2	6		3	3	10
2		3	3	6			1	6			1	10			1	51
3								1				2				3
4				1												

Grade: 0 = not observed; 1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe.

Table 4. Incidence of Splenic Nonneoplastic Lesions of Selected Severity in Male and Female Rats Exposed to Aniline Hydrochloride in the Diet for 26-104 weeks (CIIT, 1982).

Effect	Dose (mg aniline hydrochloride/kg-day)			
	0	10	30	100
Hemosiderin (severity grade 3 [moderate])	M F	M F	M F	M F
26 weeks	0/10 6/9	0/10 0/10	1/10 0/10	0/10 9/10
52 weeks	0/10 2/10	4/10* 8/10*	7/10* 10/10*	0/10 9/10*
78 weeks	3/20 6/20	8/20 20/20*	15/20* 20/20*	11/20* 16/20*
104 weeks	28/47 25/47	53/75 44/69	72/74* 64/76*	60/64* 47/77
Extramedullary hematopoiesis (severity grade 3 [moderate])	M F	M F	M F	M F
26 weeks	0/10 1/9	0/10 0/10	1/10 0/10	5/10* 2/10
52 weeks	0/10 1/10	0/10 4/10	0/10 9/10*	1/10 8/10*
78 weeks	0/20 3/20	14/20* 17/20*	20/20* 20/20*	20/20* 8/20
104 weeks	9/47 11/47	31/75* 42/69*	61/74* 57/76*	46/64* 66/77*
Congestion (severity grade 2 [slight])	M F	M F	M F	M F
26 weeks	0/10 0/9	0/10 0/10	0/10 0/10	0/10 0/10
52 weeks	0/10 0/10	0/10 0/10	0/10 0/10	0/10 0/10
78 weeks	0/20 0/20	20/20* 3/20	20/20* 17/20*	20/20* 18/20*
104 weeks	2/47 2/47	24/75* 0/69	68/74* 4/76	51/64* 75/77*
Capsulitis (severity grade 1 [minimal])	M F	M F	M F	M F
26 weeks	0/10 0/9	7/10* 5/10*	8/10* 4/10	10/10* 8/10*
52 weeks	0/10 0/10	6/10* 0/10	8/10 4/10	10/10* 10/10*
78 weeks	0/20 0/20	0/20 0/20	6/20 3/20	20/20* 18/20*
104 weeks	0/47 0/47	2/75 3/69	5/74 4/76	61/64* 64/77*

* Significantly ($p<0.05$) greater than incidence for respective control group by Fisher Exact Test performed by Syracuse Research Corporation.

12% for females. At 78 weeks, splenic parafollicular lymphoid depletion was observed in the 100 mg/kg-day groups; this progressed to splenic lymphoid atrophy at 104 weeks with an incidence of approximately 64% for males and 60% for females. Fatty metamorphosis in the splenic parenchyma was observed only in 19% of males exposed at 100 mg/kg-day for 104 weeks. Neoplastic lesions were observed after 104 weeks of treatment in areas of the spleen that developed stromal hyperplasia. Stromal sarcomas were observed in 1% of males exposed at 30 mg/kg-day and 69% of males exposed at 100 mg/kg-day. Hemangiosarcomas were observed in 3% of males and 1% of females exposed to 100 mg/kg-day. Fibrosarcomas and capsular sarcomas were observed only in males exposed at 100 mg/kg-day (incidence 3% and 1.5%, respectively).

Histological alterations were also observed in bone marrow, heart, liver, kidney, lymph nodes and adrenal glands of treated rats. The incidence of pigment deposition (presumably hemosiderin) in the hepatic sinusoids and periportal areas of the liver was increased in males and females exposed at 100 mg/kg-day for 52, 78 or 104 weeks; following exposure at 100 mg/kg-day for 104 weeks, the incidence of pigment deposition was elevated in the pancreatic lymph nodes and adrenal gland of male rats and the thoracic lymph nodes of female rats. A significant increase in the incidence of myocardial fibrosis was observed in male rats exposed to 100 mg/kg-day for 78 weeks; myocardial fibrosis and degeneration were observed in the female rats exposed to 100 mg/kg-day for 104 weeks. The authors noted that the overall incidence of cardiomyopathy (chronic myocarditis, fibrosis and degeneration) was similar in the 100 mg/kg-day female rats and the control rats. After 26 weeks of exposure, slight erythroid hyperplasia was observed in the bone marrow of male rats exposed to 100 mg/kg-day; following exposure at 100 mg/kg-day for 52 weeks, myeloid and erythroid hyperplasia were observed in both sexes and foci of cell depletion were observed in females. Increases in the proliferation of hematopoietic cells were observed in the bone marrow of rats exposed to 100 mg/kg-day for 78 or 104 weeks. These effects in the bone marrow were undoubtedly part of the regenerative response to the anemia caused by exposure to aniline.

The results of the CIIT (1982) study suggest that exposure of rats to aniline hydrochloride resulted in methemoglobin formation at a level that exceeded the capacity of methemoglobin detoxifying mechanisms in erythrocytes. Although methemoglobin levels were only statistically different from controls at ≥30 mg/kg-day, repeated exposure to aniline at ≥10 mg/kg-day resulted in significant increases in certain measures of hemolytic anemia (decreases in hemoglobin, hematocrit and erythrocyte levels) in male groups treated for 52 or 78 weeks; in females the only erythrocyte endpoint that was statistically lower compared to the controls was the erythrocyte count at 52 weeks. Increases in reticulocyte counts, generally occurring in the 30 and 100 mg/kg-day groups, but also in females treated at 10 mg/kg-day for 78 weeks, were indicative of regeneration subsequent to hemolytic anemia. The severity of extramedullary hematopoiesis in the spleen, also part of the regenerative process, was also increased in rats treated at ≥30 mg/kg-day beginning at week 26 and in the 10 mg/kg-day groups beginning at week 52. Erythroid hyperplasia was also observed in the bone marrow of rats treated at 100 mg/kg-day. The degree of accumulation of iron pigment in the spleen, resulting from its normal function as a scavenger of damaged erythrocytes, was elevated in both sexes treated at ≥10 mg/kg-day beginning at 52 weeks. At higher doses, iron pigment also accumulated in the liver, kidney, pancreatic lymph nodes and adrenal gland. In both sexes, treatment with ≥10 mg/kg-day increased the incidence of capsulitis of the spleen beginning at 26 weeks although the effect mainly persisted to termination in the 100 mg/kg-day groups; splenic capsulitis was not observed in any control rats. Congestion of the spleen was first observed in males after 52 weeks of exposure at 100 mg/kg-day, but occurred in males treated at ≥10 mg/kg-day for 78 or 104 weeks; the incidence of congestion in females was largely confined to the groups treated at ≥30 mg/kg-day for 78 weeks or 100 mg/kg-day for 104 weeks. The other splenic alterations, including lymphoid depletion and atrophy, and fatty metamorphosis, were predominantly observed in the 100 mg/kg-day groups. An analysis of incidences of splenic nonneoplastic lesions of selected severity in male and female rats exposed for 26, 52, 78, and 104 weeks shows that statistically significantly elevated incidences of splenic lesions were found in all three exposure groups compared with controls (Table 4). At the lowest exposure level, statistically significantly

increased incidences were found for: hemosiderin (of severity grade ≥ moderate) in males and females at 52 weeks and in females at 78 weeks; extramedullary hematopoiesis (of severity grade moderate) in males and females at 78 and 104 weeks; splenic congestion (of severity grade ≥ slight) in males at 78 and 104 weeks; and capsulitis (of severity grade ≥ minimal) in males at 26 and 52 weeks and females at 26 weeks (Table 4). Based on findings from this analysis, the lowest exposure level, 10 mg/kg-day, is designated as the LOAEL for adverse effects on the hematologic system and spleen in this study. Support for this designation is provided by the statistically significant findings for the 10-mg/kg-day group of decreased hemoglobin concentrations in males at 52 and 78 weeks and decreased erythrocyte counts in males at 52 and 78 weeks and females at 52 weeks (Table 1).

In a cancer bioassay, groups of 50 male and 50 female Fischer 344 rats were fed diets containing 0.3 or 0.6% of aniline hydrochloride for 103 weeks followed by a 4-week observation period (NCI, 1978). Groups of 50 male and 49 female B6C3F1 mice were fed diets containing 0.6 or 1.2% of aniline hydrochloride for 103 weeks followed by a 4-week observation period. Control groups of rats (25/sex) and mice (50/sex) were fed the basal diet throughout the study. Aniline hydrochloride doses were calculated using estimated TWA body weights (0.35 and 0.20 kg for male and female rats, respectively, and 0.040 and 0.030 kg for male and female mice, respectively) and the U.S. EPA's (1988) allometric equation for food consumption (calculated food intakes of 0.028 and 0.019 kg/day for male and female rats and 0.0067 and 0.0055 kg/day for male and female mice). For the 0.3 and 0.6% dietary concentrations, doses of 240 and 480 mg/kg-day for male rats and 285 and 570 mg/kg-day for female rats were calculated. For the mice, the 0.6 and 1.2% dietary concentrations were equivalent to doses of 1005 and 2010 mg/kg-day for males and 1100 and 2200 mg/kg-day for females. Body weights were measured twice weekly for the first 12 weeks and monthly thereafter. At the end of the study, histopathological examinations of major tissues and organs were performed. (Hematological parameters were not measured.)

In rats, no compound-related changes in mortality were observed. Decreases in body weight gain were observed in the rats exposed to 0.6% of aniline hydrochloride; the decrease was <10% in the males and 20% in the females. Non-neoplastic alterations were observed in the spleen, liver and kidneys. In the spleen, increased incidences of erythropoiesis, papillary hyperplasia, and splenic congestion were observed. The liver and kidney effects consisted of hemosiderosis observed in the renal tubular epithelium and liver Kupffer cells. The incidences of these lesions are presented in Table 5. In addition to these non-neoplastic lesions, significant increases in the incidence of hemangiosarcomas of the spleen and fibrosarcomas of the spleen and multiple organs were observed. Based on the increased incidence of alterations in the spleen and kidneys, the LOAEL is 0.3% in the diet or 240 mg/kg-day for male and 285 mg/kg-day for female rats fed aniline hydrochloride for 2 years.

Table 5. Incidence of non-neoplastic alterations in male and female rats chronically exposed to aniline hydrochloride in the diet (NCI, 1978)

Histological alteration	Male rats			Female rats		
	Control	0.3%	0.6%	Controls	0.3%	0.6%
Erythropoiesis in spleen	0/25	6/50	5/46	1/23	26/50*	30/50*
Papillary hyperplasia in spleen	0/25	18/50*	7/46*	0/23	23/50*	28/50*
Congestion in spleen	1/25	2/50	0/46	0/23	1/50	11/50*
Hemosiderosis of renal tubule cells	0/25	21/50*	34/48*	0/24	46/50*	45/50*
Hemosiderosis of hepatic Kupffer cells	0/25	2/50	26/47*	0/24	0/50	29/50*

*Statistically different from control group, $p<0.05$ (Fisher Exact Test, SRC)

No significant alterations in survival were observed in the mice (NCI, 1978). Decreases in body weight gain (approximately 10%) were observed in the male mice in the 1.2% dietary group. Histological alterations were limited to the finding of chronic inflammation of the bile ducts in male mice; the incidence was significantly higher in the 0.6 and 1.2% groups (14/49 and 13/49, respectively) than in the control group (0/39). Thus, this study identifies a LOAEL of 1005 mg/kg-day in male mice exposed to aniline hydrochloride in the diet for 2 years.

In a subchronic study conducted by Khan et al. (1993), groups of 15 male Sprague-Dawley rats were given drinking water containing 0 or 600 ppm of aniline hydrochloride for 90 days. The authors reported that the average intake of aniline hydrochloride was 60 mg/kg-day. Groups of 5 rats were killed after 30 and 60 days of exposure. No significant alterations in body weight were observed. Statistically significant increases in relative spleen weight were observed at 30, 60 and 90 days. The relative liver weight was significantly decreased at 30 days and increased at 60 days; no significant alterations in liver weight were observed at 90 days. Relative testes weight was significantly decreased at 60 days, but was comparable to control weights at 30 and 90 days. No significant alterations in relative heart, lung, kidney or brain weights were observed. The following statistically significant hematological changes were observed in the aniline-exposed rats: increased leukocyte levels at 30 days, decreased erythrocyte levels at 30, 60 and 90 days, decreased hemoglobin levels at 30 and 90 days, decreased hematocrit levels at 30 and 90 days, increased mean corpuscular volume at 60 and 90 days, increased mean corpuscular hemoglobin at 60 and 90 days, and increased methemoglobin levels at 30, 60 and 90 days. At 60 and 90 days, significant increases in IgA levels were observed; no alterations in IgM or IgG levels were noted. Splenic T-helper cell levels were decreased after 90 days of aniline exposure; no changes in splenic T cell, B cell or T-suppressor cell levels were observed (splenic lymphocyte levels were only measured at 90 days). Histological alterations

were observed in the spleen after 30, 60 or 90 days of exposure to aniline; the severity of the lesions increased with increasing exposure duration. The splenic alterations included marked red pulp expansion due to increased splenic sinusoids, fibroblasts, and macrophages, congestion of blood vessels, focal pericapsular fibrosis, and accumulation of iron in the red pulp. This study identifies a LOAEL of 60 mg/kg-day of aniline hydrochloride for damage to erythrocytes (decreased hemoglobin, hematocrit, and erythrocyte levels and iron pigment in the spleen), methemoglobinemia, splenic congestion, and possible immune effects (decreased splenic T-helper cells and IgA levels) in male rats exposed to aniline hydrochloride in the diet for 30-90 days.

In a pilot study conducted for CIIT (1977), groups of 10 male and 10 female Fischer 344 rats were exposed to 0, 30, 100, 300 or 1000 mg/kg-day of aniline hydrochloride in the diet for 4 weeks. Animals in the 1000 mg/kg-day group were sacrificed after 24-27 days of exposure due to a high morbidity rate. Clinical observations included paleness of the eyes, ears, front paws and hind paws in the 300 and 1000 mg/kg-day groups and thinness and a hunched appearance in the 1000 mg/kg-day group. Food consumption and body weight gain were reduced in the 1000 mg/kg-day group compared to the controls. Statistically significant increases in methemoglobin, reticulocytes and Heinz bodies were observed in all groups of rats exposed to aniline hydrochloride; the increases were dose-related except there was a drop-off in the value for Heinz bodies in females exposed at 1000 mg/kg-day compared to those exposed at 300 mg/kg-day . Additional hematological parameters were not measured. Darkened or black spleens, livers, and kidneys were observed in the groups exposed at 300 mg/kg-day or higher. The study design did not include histological examination of tissues. This study identifies a LOAEL of 30 mg/kg-day of aniline hydrochloride for erythrocyte toxicity (increases in methemoglobinemia, Heinz bodies and reticulocytes) in rats exposed to dietary aniline hydrochloride for 4 weeks.

The potential of aniline to induce developmental effects has been examined in rats and mice (Jones-Price et al., 1981; Price et al., 1985; Piccirillo et al., 1983; Hardin et al., 1987). Prenatal exposure to aniline does not result in malformations or anomalies or alterations in number of live offspring, birth weight or weight gain. Impaired pup survival has been observed in rats (not statistically significant) (Jones-Price et al., 1981; Price et al., 1985) and mice (Piccirillo et al., 1983; Hardin et al., 1987).

In a two-part developmental toxicity study by Jones-Price et al. (1981; Price et al.,1985), groups of pregnant Fischer 344 rats (21-24 dams per group) were dosed via gavage with 0, 10, 30 or 100 mg/kg-day of aniline hydrochloride in water on gestational days 7-20 (study A) or on gestational day 7 through postnatal day 0 (study B). In study A, the rats killed on gestational day 20 showed significant alterations in relative maternal liver weight, gravid uterine weight, average placental weight, number of live fetuses, percentage of live male fetuses per litter, average fetal body weight per litter, crown-rump length per litter, or relative fetal spleen weight per litter. A dose-related decrease in maternal absolute weight gain (i.e., weight gain during gestation minus gravid uterine weight) was statistically significant at the 100 mg/kg-day dose. Significant increases in relative spleen weight were observed in dams treated with $\geq$10 mg/kg-day. Significant increases in methemoglobin, reticulocyte and leukocyte levels and mean corpuscular volume, and decreases in erythrocyte levels, were observed in the dams treated with 100 mg/kg-day. In the 100 mg/kg-day group, there were significant increases in fetal relative liver weights

and the erythrocyte distribution width in fetuses. However, no significant alterations in the incidence of external, visceral or skeletal malformations or variations were observed in the aniline-exposed fetuses. In study A, 10 mg/kg-day was a LOAEL for maternal toxicity (increased relative spleen weight); the developmental NOAEL was 30 mg/kg-day and the LOAEL was 100 mg/kg-day for increased relative liver weights in fetuses. (Since this study did not evaluate hematology parameters in the low- or mid-dose groups, it is not certain whether 30 mg/kg-day would have been a NOAEL for increased erythrocyte width distribution in fetuses.)

In study B by Jones-Price et al. (1981; Price et al., 1985), the rat dams treated with aniline on gestational day 7 through postnatal day 0 were killed on postnatal day 30 (PND 30). At birth, litters were culled to a maximum of eight pups. Pup body weights were recorded on PND 2, 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 35, 40, 50 and 60. Most pups were sacrificed on PND 60, but one pup was randomly selected from each litter for sacrifice on postnatal days 0, 10, 25, and 50 to collect blood samples and measure the weights of the liver weights and spleen. Methemoglobin levels and other hematology parameters were measured only in control and 100 mg/kg-day groups of pups and dams. Pups were evaluated for the appearance of neurobehavioral and developmental landmarks up to PND 60. On postnatal day 30, significant increases in relative spleen weight, methemoglobin levels and mean corpuscular volume were observed in the 100 mg/kg-day dams. No significant alterations in body weight gain or relative liver weights were observed in dams. On postnatal day 0, no significant alterations in live litter size, incidence of stillborn pups, percentage of male pups per litter, pup weight or length, or pup relative spleen or liver weights were observed. Mortality rates were 8.3, 9.6, 20.8 and 12.5% in the pups of rats administered 0, 10, 30 or 100 mg/kg-day, respectively; the number of affected litters was 2/15, 3/16, 4/15 and 5/16, respectively. At postnatal day 2, body weights of the female pups in the 100 mg/kg-day group were significantly lower than in the control group; no significant alterations in body weight were observed at postnatal day 10, 25, 50 or 60. Relative liver weights were significantly increased in the 10 and 25 mg/kg-day groups at postnatal day 25 and in the 10 mg/kg-day offspring at postnatal day 50; no significant alterations were observed at postnatal day 10 or 60 or in the 100 mg/kg-day group. No significant alterations in relative spleen weights were observed in the offspring. The only alteration in hematological parameters observed in the offspring was a significant increase in mean corpuscular volume in the 100 mg/kg-day group on postnatal day 0. Physical development (pinna detachment, visible pilation, lower incisor eruption, eye opening, vaginal opening, and testis descent), behavioral development (surface righting, cliff avoidance, auditory startle, wire grasping, and mid-air righting) and behavior in open field test were not affected by prenatal exposure to aniline hydrochloride. This rat developmental toxicity study identifies a LOAEL of 10 mg/kg-day for maternal toxicity (increased spleen weight) and a NOAEL of 30 mg/kg-day and a LOAEL of 100 mg/kg-day for developmental toxicity (decreased body weight at postnatal day 2). (As in study A, there is uncertainty as to the no-effect levels for hematological effects (increased erythrocyte distribution width and increased mean corpuscular volume) observed in offspring at 100 mg/kg-day because the 10 and 30 mg/kg-day dose groups were not evaluated.)

Piccirillo et al. (1983; Hardin et al., 1987) administered 0 or 560 mg/kg-day of aniline in corn oil to groups of 25 pregnant CD-1 mice on gestational days 7-14. Decreased maternal weight gain was observed during gestational days 7-18; however no differences in body weight were observed. No effects on the time to deliver were observed. Pup birth weight and weight

gain at postnatal day 3 were significantly lower in the aniline-exposed group. Decreases in pup viability, as evidenced by significant increases in the number of dead pups at postnatal day 3, were observed. Thus, this study identifies a minimal LOAEL of 560 mg/kg-day for maternal effects and a FEL of 560 mg/kg-day for decreased offspring viability in mice administered aniline on gestational days 7-18.

DERIVATION OF A PROVISIONAL CHRONIC RfD FOR ANILINE

Based on the available data on the toxicity of aniline, the primary targets of toxicity appear to be the erythrocyte and the spleen (CIIT, 1982; Khan et al., 1993). Increased levels of methemoglobin were observed in humans exposed to an oral dose of 25 mg aniline (0.36 mg/kg); no effects on methemoglobin were observed at 15 mg (Jenkins et al., 1972). In rats, decreased hemoglobin, hematocrit and erythrocyte levels were observed following dietary exposure to $\geq$10 mg/kg-day of aniline hydrochloride (CIIT, 1982). At higher concentrations ($\geq$30 mg/kg-day of aniline hydrochloride), increased reticulocyte, Heinz bodies and methemoglobin levels were observed (CIIT, 1982). Statistically significant increases in the incidences of nonneoplastic lesions (capsulitis, congestion, extramedullary hematopoiesis and hemosiderin deposition) were observed in the spleens of rats that ingested $\geq$10 mg/kg-day of aniline hydrochloride. At higher doses of aniline hydrochloride ($\geq$100 mg/kg-day), lymphoid depletion and atrophy, fatty metamorphosis, stromal hyperplasia, and stromal sarcomas were observed in the spleen of rats (CIIT, 1982; NCI, 1978). It is likely that stromal hyperplasia is a pre-neoplastic lesion. Hemosiderin deposition was also observed in the liver, kidneys, pancreatic lymph nodes and adrenal glands in rats dietarily exposed to $\geq$100 mg/kg-day of aniline hydrochloride (CIIT, 1982; NCI, 1978). A no-effect level was not identified in long term animal studies.

The LOAEL of 10 mg/kg-day of aniline hydrochloride for hematological and splenic effects in rats identified by the CIIT (1982) study was selected as the basis of the p-RfD for aniline. Since reversible hematological effects were reported in rats exposed to 10 mg aniline hydrochloride kg-day (CIIT, 1982), this dosage could be considered as a minimal LOAEL. The NOAEL and LOAEL (0.2 and 0.36 mg/kg) in humans from the Jenkins et al. (1972) study were not selected as the basis of the p-RfD because it is a single exposure study examining a limited number of endpoints with a short observation period (4 hours). The 10 mg/kg-day dose of aniline hydrochloride is converted into an equivalent dose of aniline by multiplying by the ratio of the aniline molecular weight (93.12) to the aniline hydrochloride molecular weight (129.57). This minimal LOAEL of 7 mg/kg-day is divided by an uncertainty factor of 1000 (3 to extrapolate from a LOAEL, 10 to account for interspecies extrapolation, 10 for human variability and 3 to account for lack of a reproductive and multigenerational developmental study) to yield a **chronic p-RfD of 7E-3 mg/kg-day for aniline.** (The p-RfD for aniline hydrochloride would be 1E-2 mg/kg-day.)

Jenkins et al. (1972) noted that the results of their study suggest that humans are more sensitive to the toxicity of aniline than rats. In its derivation of an RfC for this chemical, IRIS (U.S. EPA, 2007) noted that the reason for the increased sensitivity in humans to methemoglobin production is not known, but it is not likely due to methemoglobin turnover since methemoglobin half life is three times longer in rats as compared to humans. However, the

capacity of methemoglobin reductase in erythrocytes is five times higher in rats than in humans (Smith, 1995). The p-RfD of 7E-3 mg/kg-day based on rat data is thirty times lower than the human NOAEL (0.21 mg/kg-day) and fifty times lower than the human LOAEL (0.36 mg/kg-day) identified in the Jenkins et al. (1972) study. Therefore, exposures to aniline at the p-RfD are unlikely to elicit signs of toxicity in humans.

Confidence in the principal study, CIIT (1982), is medium-to-high. It is a well designed study examining a number of relevant endpoints using an adequate number of male and female animals with several interim sacrifices, however, it failed to explain the omission of histopathological data for some animals (~20 per sex) in the control groups at terminal sacrifice. Confidence in the database is medium. The toxicity of aniline has been tested in 2 chronic rat studies (CIIT, 1982; NCI, 1978), a chronic mouse study (NCI, 1978), two subchronic rat studies (Khan et al., 1993; CIIT, 1977), and developmental toxicity studies in rats and mice. Confidence is medium because the database lacks a two-generation reproductive toxicity study. Reflecting the medium confidence in the database, confidence in the p-RfD is medium.

DERIVATION OF A PROVISIONAL CHRONIC RfC FOR ANILINE

The availability of a chronic RfC for aniline on IRIS (U.S. EPA, 2007) precludes review of pertinent noncancer toxicity data for inhalation exposure. No studies were located in review documents (U.S. EPA, 1985, 1992; IARC, 1974, 1982; Weisburger and Hudson, 2001) or in the literature search regarding carcinogenicity of inhaled aniline to animals.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR ANILINE

IRIS (U.S. EPA, 2007) has classified aniline as a probable human carcinogen (Group B2). No OSFs were developed because an OSF exists on IRIS. IRIS does not provide an IUR, but development was not attempted here because there is no new information and we are not comfortable with the route to route extrapolation from the CIIT data (CIIT, 1982; NCI, 1978) which was attempted by HEED (U.S. EPA, 1992).

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