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Provisional Peer Reviewed Toxicity Values for

N-Methylaniline
(CASRN 100-61-8)

Derivation of Subchronic and Chronic Oral RfDs

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
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
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

8-3-05

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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.

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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

An RfD for N-methylaniline is not listed on IRIS (U.S. EPA, 2005), the HEAST (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002). The CARA list (U.S. EPA, 1991a, 1994) does not include any documents for N-methylaniline. ATSDR (2003), IARC (2003) and WHO (2003) have not published toxicological reviews of this compound. A toxicity review on aromatic amines (Weisburger and Hudson, 2001) and the NTP (2003a,b) management status and health and safety reports were consulted for relevant information. Literature searches were conducted in TOXLINE (1965-1992), CANCERLINE (1963-1992), CHEM ID, HSDB, RTECS and TSCATS in May, 1992. Update computer literature searches were conducted in TOXLINE (1992-1994), MEDLINE (1992-1994), CANCERLINE (1992-1994), TSCATS and RTECS in March, 1994. Finally, update literature searches were screened for the period from 1994 to January, 2003 in the following databases:

TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. Additional literature searches from January 2003 through October 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases

REVIEW OF PERTINENT LITERATURE

Human Studies

No relevant data were located regarding the toxicity of N-methylaniline to humans following oral exposure.

Animal Studies

Oral toxicity studies for N-methylaniline in animals include two carcinogenicity assays and one 28-day gavage assay in rats. Chronic studies (Sander, 1971; Greenblatt et al., 1971) had several experimental uncertainties and provided no information useful for deriving a p-RfD for N-methylaniline.

Sander (1971) administered N-methylaniline in feed to 16 female rats (strain SIV 50) at a dietary concentration of 0.09% for 114 days; using U.S. EPA (1988) reference values for body weight and food consumption, the dose is calculated as 102 mg/kg-day. Control rats (n=36) received standard diets. Both groups were maintained on standard diets for an additional 669 days. All rats were necropsied and suspected neoplastic tissues were examined histologically. Treatment with N-methylaniline had no adverse effect on survival or tumor incidence. No information was provided about non-neoplastic effects in tissues.

In a preliminary study for a pulmonary adenoma assay, Greenblatt et al. (1971) administered N-methylaniline at a dietary concentration of 7.8 g per kg food to Swiss mice. Using reference values for body weight and food consumption in U.S. EPA (1988), the dose is calculated as 1457 mg/kg-day. In the main study, N-methylaniline was administered at a dietary concentration of 1.95 g per kg food to Swiss mice (20/sex) for 28 weeks, after which the mice received control diets for 12 weeks. As above, the dose in the main study is calculated as approximately 364 mg/kg-day for male and female mice. Controls received standard diets for 40 weeks. Aside from a comment that the high dose of 1457 mg/kg-day in the preliminary study produced severe cyanosis, presumably due to methemoglobinemia, no information was provided about non-neoplastic effects in this study.

A description of a 4-week gavage assay conducted in Japan was available as a brief summary in English (GINC, 2003); the more complete report in Japanese includes data tables in

English (Biosafety Research Center, undated). In addition, the complete report of the Japanese study was translated in English and evaluated by the NCEA-Cincinnati scientists. In this Japanese study, N-methylaniline at doses of 0, 5, 25 or 125 mg/kg-day was administered by gavage in corn oil to groups of CD rats (5/sex) for 28 days. Hematology, urinalysis and clinical chemistry data were collected before terminal sacrifice on day 29. At termination, all rats were necropsied, and data were collected for body weights and organ weights (brain, liver, kidneys, spleen, adrenals, and testes or ovaries). The liver, kidney, spleen, bone marrow, adrenal and femur were evaluated for histopathology.

Treatment with N-methylaniline had no adverse effect on survival or body weights. Dose-related changes in hematology, serum chemistry and organ weights are shown in **Table 1**. Alterations in indicators of erythrocyte toxicity (reduced hematocrit, hemoglobin and erythrocyte counts) and compensatory hematopoiesis (elevated reticulocyte counts) were observed in both sexes at $\geq$25 mg/kg-day in a dose-related fashion; in addition, hemoglobin was significantly reduced in females treated at 5 mg/kg-day. Although methemoglobin levels were not reported in the Japanese study (Biosafety Research Center, undated), evidence from the inhalation study (Markosyan, 1969), as well as chronic oral toxicity data on the related compound aniline (CIIT, 1982), implicate methemoglobin production as the primary effect of N-methylaniline exposure. Statistically significant ($p<0.05$) serum chemistry changes included increased total bilirubin in both sexes at 125 mg/kg-day and slight increases in AST in males at 125 mg/kg-day and creatinine in females at $\geq$5 mg/kg-day. No significant urinalysis parameters were identified, except that urine volume was higher and urine color was darker in the groups treated at 125 mg/kg-day. Absolute and relative spleen weights were significantly elevated in males at 125 mg/kg-day and females at $\geq$25 mg/kg-day. Significant gross necropsy findings included splenomegaly (all males at $\geq$25 mg/kg-day; 3/5 females at 25 mg/kg-day and 5/5 females at 125 mg/kg-day) and black coloration (presumably hemosiderin, possibly methemoglobin, deposition) of the spleen (all rats at $\geq$25 mg/kg-day), liver (all rats at 125 mg/kg-day), and kidney (4/5 males and 5/5 females at 125 mg/kg-day). No gross findings were observed in the controls. Histopathological changes were observed in the spleen (congestion in all males at $\geq$5 mg/kg-day and all females at $\geq$25 mg/kg-day; pigment deposition and increased hematopoiesis in all rats at $\geq$25 mg/kg-day), bone marrow (increased hematopoiesis in 4/5 females at 25 mg/kg-day and all males and females at 125 mg/kg-day), liver (pigment deposition and extramedullary hematopoiesis in all rats at 125 mg/kg-day), and kidney (pigment deposition in all rats at 125 mg/kg-day). These changes were all considered to be of slight severity, even at the highest dose group, and, with the exception of pigment deposition in the spleen of 2/5 females, were not seen in controls at all. In addition to these lesions, male rats showed a dose-related increase in incidence and severity of hyaline droplet formation in the kidney. This lesion, however, is characteristic of a male-rat-specific nephropathy associated with the presence of a low molecular weight protein, $\alpha_{2\mu}$-globulin, in male rats, and is not considered to be predictive of a renal effect in humans (U.S. EPA, 1991b).

Table 1. Dose-Related Changes in Rats Exposed to N-Methylaniline by Gavage for 28 Days: A) Hematology, B) Serum Chemistry, C) Organ Weight (Japanese Study, undated; GINC, 2003)								
	Male rats				Female rats			
Dose mg/kg-day	Control	5	25	125	Control	5	25	125
A) Hematology								
Hematocrit (%)	45.3	44.2	40.7*	33.7**	44.6	43.3	39.9**	35.6**
Hemoglobin (g/dL)	15.0	14.6	13.2*	11.8**	14.5	13.9*	12.7**	12.3**
Erythrocytes (x 10^6/mm^3)	7.30	7.10	6.37*	4.37**	7.02	7.12	6.20**	4.56**
Reticulocytes (%)	43	58	113**	943**	26	36	125*	639**
B) Serum chemistry								
AST (U/L)	54	50	46	75**	59	55	71	67
Creatinine (mg/dL)	0.52	0.51	0.50	0.53	0.52	0.68**	0.66**	0.71**
Total bilirubin (mg/dL)	0.11	0.12	0.16	0.77**	0.14	0.15	0.20	0.44**
C) Organ weight								
Spleen absolute weight (g)	0.54	0.56	0.84	3.37**	0.36	0.39	0.67*	1.60**
Spleen relative weight (%)	0.193	0.172	0.261	1.088**	0.183	0.203	0.320*	0.804**
Significant difference from control group: * $P \leq 0.05$; ** $P \leq 0.01$								

Although some minor effects were noted at 5 mg/kg-day (Biosafety Research Center, undated), the weight of evidence of the hematology, organ weight, and pathology data suggests that adverse effects occurred at 25 mg/kg-day and above. Hemoglobin was slightly reduced in females at 5 mg/kg-day, but without concommitant changes in hematocrit and erythrocyte count. By contrast, all of these measures were significantly reduced in both males and females at 25 mg/kg-day. Creatinine levels were elevated in all dosed female rats. Slight splenic congestion was observed in males at 5 mg/kg-day, but other lesions, more clearly associated with the hematological effects of the chemical (splenomegaly; pigment deposition and increased hematopoiesis in the spleen, bone marrow, liver and kidney) were increased only at 25 mg/kg-day and above. Therefore, this study identified a LOAEL of 25 mg/kg-day and NOAEL of 5 mg/kg-day for splenic and hematological effects.

Other Studies

Systemic effects of oral exposure to N-methylaniline are similar to those following inhalation exposure. Rats continuously exposed by inhalation at a concentration of 0.3 mg/m^3 for

100 days exhibited increased methemoglobin levels, decreased numbers of erythrocytes, and secondary effects in the spleen (hematopoiesis, splenomegaly) (Markosyan, 1969).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR N-METHYLANILINE

Based on the available data for N-methylaniline, the primary targets of toxicity appear to be the erythrocyte and the spleen (Biosafety Research Center, undated; GINC, 2003). Although methemoglobin levels were not measured in this study, evidence from the subchronic inhalation test on N-methylaniline (Markosyan, 1969), as well as chronic oral toxicity data on the related compound aniline (CIIT, 1982), implicate methemoglobin production as the primary effect of N-methylaniline exposure. In rats exposed to N-methylaniline by gavage for 28 days, minimal effects on erythrocytes (significantly reduced hemoglobin in females) and slight congestion of the spleen in males were observed at the NOAEL of 5 mg/kg-day. At the LOAEL of 25 mg/kg-day, significant evidence of anemia (reduced hematocrit, hemoglobin and erythrocyte counts), as well as compensatory hematopoiesis was observed in both sexes. Pigment deposition in several tissues at 125 mg/kg-day was presumably related to erythrocyte destruction.

The NOAEL of 5 mg/kg-day for hematological and splenic effects in rats identified in the study by the Biosafety Research Center (undated; GINC, 2003) was selected as the basis for the subchronic RfD for N-methylaniline. An uncertainty factor of 300 was derived, consisting of factors of 10 for extrapolation from rats to humans, 10 to protect sensitive individuals, and 3 for deficiencies in the database, including absence of supporting chronic or subchronic studies and any reproduction or developmental studies). Application of the composite uncertainty factor of 300 to the NOAEL of 5 mg/kg-day yields a provisional **subchronic RfD of 2E-2 mg/kg-day** for N-methylaniline.

subchronic p-RfD = NOAEL / UF
= 5 mg/kg-day / 300
= 0.02 mg/kg-day or 2E-2 mg/kg-day

The chronic p-RfD is similarly derived by applying a composite uncertainty factor of 3000 to the NOAEL of 5 mg/kg-day. The composite uncertainty factor includes a factor of 300, as derived above for the subchronic p-RfD, and an additional factor of 10 for use of a subchronic study. Application of the uncertainty factor of 3000 to the NOAEL of 5 mg/kg-day yields a provisional **chronic RfD of 2E-3 mg/kg-day** for N-methylaniline.

p-RfD = NOAEL / UF
= 5 mg/kg-day / 3000
= 0.002 mg/kg-day or 2E-3 mg/kg-day

Confidence in the critical study is low. The study included investigation of a broad array of systemic endpoints in multiple dose groups and identified a NOAEL and LOAEL. Tabular results were well reported. The study was limited by small group sizes, short duration, and failure to analyze for methemoglobin. Confidence in the database is low because of the lack of supporting systemic toxicity data and developmental and reproductive toxicity studies. Confidence in the subchronic and chronic p-RfDs for N-methylaniline is, therefore, low.

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10-04-2005

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HEC	human equivalent concentration
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p-RfC	**provisional inhalation reference concentration**
p-RfD	**provisional oral reference dose**
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INTRODUCTION

An RfC for N-methylaniline is not listed on IRIS (U.S. EPA, 2005) or the HEAST (U.S. EPA, 1997). ACGIH (2001, 2002) and NIOSH (2003) established occupational exposure limits (8-hour TWA) of 0.5 ppm (2.2 mg/m^3) for N-methylaniline to protect against methemoglobinemia and its sequelae (anoxia, cyanosis, dizziness, weakness and headache). OSHA (2003a,b) had proposed an identical PEL in its final rule of 1989, but after this rule was revoked in 1993, the PEL reverted to its former value of 2 ppm (9 mg/m^3). All three agency limit values include a skin notation to indicate that dermal absorption of N-methylaniline can contribute significantly to systemic toxicity. The CARA list (U.S. EPA, 1991, 1994) does not include any documents for N-methylaniline. ATSDR (2003), IARC (2003) and WHO (2003) have not published toxicological reviews of this compound. A toxicity review on aromatic amines (Weisburger and Hudson, 2001) and the NTP (2003a,b) management status and health

and safety reports were consulted for relevant information. Literature searches were conducted in TOXLINE (1965-1992), CANCERLINE (1963-1992), CHEM ID, HSDB, RTECS and TSCATS in May, 1992. Updated computer literature searches were also conducted in TOXLINE (1992-1994), MEDLINE (1992-1994), CANCERLINE (1992-1994), TSCATS and RTECS in March, 1994. Additional literature searches were screened for the period from 1994 to September 2005.

REVIEW OF PERTINENT LITERATURE

Human Studies

No relevant data were located regarding the toxicity N-methylaniline to humans following inhalation exposure.

Animal Studies

No chronic inhalation studies in animals were located for N-methylaniline. The literature search uncovered one subchronic study in rats.

In the subchronic inhalation study, rats were exposed to 0.04 or 0.3 mg/m^3 of N-methylaniline or to 0.005 or 0.3 mg/m^3 of the related compound N,N-dimethylaniline continuously for 100 days (Markosyan, 1969). No information on the number or strain of animals, treatment of controls, purity of the compounds, generation of the exposure atmosphere or monitoring of the exposure level was provided. Although statistical significance is mentioned in the report, no information on statistical methods was provided. For both compounds, effects at the highest exposure level included increased methemoglobin levels, reduced erythrocyte counts and hemoglobin levels, reticulocytosis, leukopenia, splenomegaly and splenic hemosiderosis. For the most part, no documentation was provided for these effects. A number of other changes in serum or tissue constituents were also reported. Some histological changes were reported in the brain, but these could reflect autolysis during the interval prior to fixation. Histological changes in the livers and lungs were also reported. No effects were seen at the lower exposure levels. Given the lack of information regarding experimental methods and results, and the lack of corroborating data from the literature, this study is not a suitable basis for deriving an RfC. Single dose exposure studies conducted in dogs, cats, rabbits, rats and guinea pigs indicated a range of exposure (2.3 to 7.6 ppm) considered safe for exposure to N-methylaniline in most sensitive species (ACGIH, 2001). However, methemoglobinemia and formation of Heinz bodies have been reported at 7.6 ppm and 2.4 ppm, respectively (U.S. EPA, 2005).

Other Studies

No adequate compound-specific data are available for the inhalation toxicity of N-methylaniline to derive a p-RfC.

The conversion of hemoglobin to methemoglobin is one of the characteristic effects caused by metabolites of aromatic amines (Weisburger and Hudson, 2001). N-methylaniline (U.S. EPA, 2005) has been shown to induce methemoglobin formation in erythrocytes of exposed animals (Markosyan,1969; Oberst et al., 1956). The sequelae of methemoglobin formation are: erythrocyte effects (reduced hemoglobin, hematocrit and erythrocyte counts), cyanosis from the reduced oxygen-carrying capacity of the blood, and secondary splenic effects (hemosiderosis, hematopoiesis, splenomegaly) (Markosyan, 1969; Greenblatt et al., 1971; GINC, 2003; Biosafety Research Center, undated a; U.S. EPA, 1992, 2005). In a 4-week gavage study in rats exposed to N-methylaniline, the NOAEL was 5 mg/kg-day and the LOAEL was 25 mg/kg-day for blood and spleen effects (GINC, 2003; Biosafety Research Center, undated b).

Several different phase I metabolic pathways in the liver have been identified for aniline (U.S. EPA, 1992): N-acetylation, aromatic hydroxylation and N-hydroxylation. The acetylation of aniline by N-acetyltransferase results in the formation of acetanilide, which is further metabolized to products excreted in urine. In organisms with efficient acetylation processes (mice and humans with a ‘fast acetylator’ phenotype), a smaller percentage of an administered dose of aniline is metabolized by the other two pathways, which are known to generate reactive intermediates (Weisburger and Hudson, 2001). The most toxicologically significant pathway is N-hydroxylation of aniline by cytochrome P450 (CYP450) mixed function oxidases to form phenylhydroxylamine. In erythrocytes, the metabolism of phenylhydroxylamine to nitrosobenzene results in the conversion of hemoglobin to methemoglobin (U.S. EPA, 1992). CYP450 enzymes also catalyze ring hydroxylation of aniline to aminophenols, which may be subsequently conjugated to form glucuronide or sulfate metabolites typically excreted in urine. The metabolism of N-methylaniline is expected to be similar to aniline following an N-demethylation reaction, which is a function of heme-containing enzymes such as CYP450 and peroxidases (Hover and Kalkami, 2000). N-demethylation of N-methylaniline has been demonstrated in erythrocytes (Stecca et al., 1992). N-glucuronidation has been reported in primary cultures of rat hepatocytes treated with N-methylaniline (Sherrat and Damani, 1989). In the acidic conditions of the stomach, N-methylaniline reacts with nitrite to form N-nitroso derivatives (Greenblatt et al., 1971; Sander, 1973). However, because of considerable difference in solubility in water, the metabolic pathway for N-methylaniline may not be similar to aniline in assessing critical effects via inhalation route of exposure.

DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC RFCs FOR N-METHYLANILINE

Inhalation data for N-methylaniline are limited to one study (Markosyan, 1969), which has several uncertainties in study design, data collection and data analysis; information pertinent to mode of action and route-specific toxicodynamics are not available. Lack of route-specific pharmacokinetic/pharmacodynamic information precludes derivation of either subchronic or chronic p-RfCs. The Markosyan (1969) study did not provide adequate dose-response data on methemoglobinemia. Therefore, additional studies are needed in support of this critical effect.

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9-20-2005

Provisional Peer Reviewed Toxicity Values for

N-Methylaniline
(CASRN 100-61-8)

Derivation of a Carcinogenicity Assessment

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
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
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

9-20-2005

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR N-METHYLANILINE (CASRN 100-61-8) Derivation of a Carcinogenicity Assessment

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 science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. 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 cancer assessment for N-methylaniline is not listed on IRIS (U.S. EPA, 2005a), the HEAST (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002). The CARA list (U.S. EPA, 1991, 1994) does not list any documents for N-methylaniline. ATSDR (2003), IARC (2003) and WHO (2003) have not published toxicological reviews of this compound. A toxicity review on aromatic amines (Weisburger and Hudson, 2001) and the NTP (2003a,b) management status and health and safety reports were consulted for relevant information. Literature searches were conducted in TOXLINE (1965-1992), CANCERLINE (1963-1992), CHEM ID, HSDB, RTECS and TSCATS in May, 1992. Updated computer literature searches were also conducted in TOXLINE (1992-1994), MEDLINE (1992-1994), CANCERLINE (1992-1994), TSCATS and RTECS in March, 1994. Additional literature searches were screened for the period from 1994 to September 2005.

REVIEW OF PERTINENT LITERATURE

Human Studies

No studies were located regarding the carcinogenicity of N-methylaniline to humans by any route of exposure.

Animal Studies

Two oral carcinogenicity studies in rodents are available for N-methylaniline, but neither is adequate by current standards. No inhalation carcinogenicity studies in animals were located.

Sander (1971) evaluated the carcinogenicity of N-methylaniline and related compounds in rodents to evaluate the effect of nitrosamine formation. A group of 16 female rats (strain SIV 50) were given 0.09% N-methylaniline in the diet for 114 days; using reference values for body weight and food consumption in U.S. EPA (1988), the dose is calculated as 102 mg/kg-day. A control group of 32 rats received a standard diet and untreated drinking water. Three other groups received drinking water containing 1.0% sodium nitrite and diets with or without N-methylaniline (0.015 or 0.03%) for 114-117 days; as above, the doses of N-methylaniline are calculated as 17 or 24 mg/kg-day. All groups received standard diets and untreated drinking water for the remaining study period. The study was terminated on day 783, by which time all rats in the dually-treated groups had died. All rats were necropsied and suspect tissues were examined histologically. Treatment with N-methylaniline alone or sodium nitrite alone had no adverse effect on survival or the incidence of tumors. However, simultaneous administration of sodium nitrite and N-methylaniline significantly increased mortality and the incidence of tumors, notably in the esophagus (adenomas and carcinomas) and nasal cavity (aesthesioneuroepithelioma and carcinoma). Carcinogenicity was attributed to the formation of the N-nitroso derivative of N-methylaniline (Sander, 1973). This study is not an adequate test of the carcinogenicity of N-methylaniline alone because the period of exposure was relatively short, the group sizes were small, a single dose level was tested (without co-exposure to nitrite), and the histological examination appears to have been limited to ‘interesting’ tissues.

Similar results were reported in a 40-week pulmonary adenoma assay by Greenblatt et al. (1971). N-methylaniline at a dietary concentration of 1.95 g per kg was administered to Swiss mice (20/sex) for 28 weeks, after which the mice received standard diets for 12 weeks. Using reference values for food consumption and body weight in U.S. EPA (1988), the dose is calculated as 364 mg/kg-day for male and female mice. A control group of 80/sex received standard diets for 40 weeks. At termination, mice were necropsied and examined for lung adenomas exceeding 0.5 mm; the diagnosis was confirmed microscopically in every fifth animal. Treatment with N-methylaniline alone did not increase the incidence of pulmonary adenoma. The incidence of malignant melanoma at 40 weeks was higher in the treated group compared to

the control group (14% versus 7%). A third group that was given 1.95 g N-methylaniline per kg food and drinking water containing 0.1% sodium nitrite for 28 weeks showed a significant increase in the incidence of pulmonary adenoma; this result was attributed to the production of nitrosamine. This study is not an adequate test of the carcinogenicity of N-methylaniline alone because histopathology analysis was limited to the lung and because the group sizes were small, a single dose level was tested (without co-exposure to nitrite), and the study duration was intentionally truncated to facilitate the counting of individual lung tumors.

Other Studies

Whereas little information is available on the metabolism of N-methylaniline, several different phase I metabolic pathways in the liver have been identified for aniline (U.S. EPA, 1992): N-acetylation, aromatic hydroxylation and N-hydroxylation. The acetylation of aniline by N-acetyltransferase results in the formation of acetanilide, which is further metabolized to products excreted in urine. In organisms with efficient acetylation processes (mice and humans with a 'fast acetylator' phenotype), a smaller percentage of an administered dose of aniline is metabolized by the other two pathways, which are known to generate reactive intermediates (Weisburger and Hudson, 2001). The most toxicologically significant pathway is N-hydroxylation of aniline by cytochrome P450 (CYP450) mixed function oxidases to form phenylhydroxylamine. In erythrocytes, the metabolism of phenylhydroxylamine to nitrosobenzene results in the conversion of hemoglobin to methemoglobin (U.S. EPA, 1992). CYP450 enzymes also catalyze ring hydroxylation of aniline to aminophenols, which may be subsequently undergo phase II conjugation reactions to form glucuronide or sulfate metabolites typically excreted in urine. The metabolism of N-methylaniline is expected to be similar to aniline following an N-demethylation reaction, which is a function of heme-containing enzymes such as CYP450 and peroxidases (Hover and Kalkami, 2000). N-demethylation of N-methylaniline has been demonstrated in erythrocytes (Stecca et al., 1992). N-glucuronidation has been reported in primary cultures of rat hepatocytes treated with N-methylaniline (Sherrat and Damani, 1989). In the acidic conditions of the stomach, N-methylaniline reacts with nitrite to form N-nitroso derivatives (Greenblatt et al., 1971; Sander, 1973).

The acute oral toxicity of N-methylaniline in rats is similar to that of aniline. The acute oral LD50 values for N-methylaniline were 782 and 716 mg/kg in males and females, respectively (GINC, 2003; Biosafety Research Center, undated a). Acute oral LD50 values in several tests for aniline ranged between 440 and 1072 mg/kg for the free base and 840 to 1070 for the hydrochloride salt (U.S. EPA, 1992; Health Canada, 1994).

A characteristic effect of the biotransformation of aromatic amines such as N-methylaniline and aniline is the formation of methemoglobin, which can occur independent of the route of exposure (Weisburger and Hudson, 2001). N-methylaniline and aniline have been shown to induce methemoglobin formation in erythrocytes of exposed animals, resulting in

hemolytic anemia and secondary splenic effects (hemosiderosis, hematopoiesis, splenomegaly) (Markosyan, 1969; U.S. EPA, 2003a). Evidence for methemoglobinemia has also been provided in some studies in which methemoglobin was not measured. In a preliminary test for the pulmonary adenoma assay described above, rats receiving a dietary concentration of 7.8 g/kg per kg (dose calculated as approximately 1457 mg/kg-day) developed cyanosis, which is a typical effect of methemoglobinemia (Greenblatt et al., 1971). Erythrocyte effects (reduced hematocrit, hemoglobin and erythrocyte counts) and splenic effects (increased organ weight, splenomegaly, pigmentation and hematopoiesis) were observed in rats exposed to N-methylaniline by gavage for 28 days (GINC, 2003; Biosafety Research Center, undated b).

In evaluating common features of carcinogenicity caused by aromatic amines, Goodman et al. (1984) concluded that both genotoxic and non-genotoxic mechanisms were possible; both mechanisms involve biotransformation by CYP450. The genotoxic mechanism relates to the generation of reactive intermediates during metabolism and is presumably the cause of carcinogenicity in the liver and other tissues. A non-genotoxic mechanism was proposed for rare tumors of the spleen, in which accumulation of erythrocytes damaged from methemoglobin formation would lead to splenic fibrosis, and subsequently, to splenic tumors. However, this mechanism cannot rule out the possibility that reactive metabolites are transferred from the damaged erythrocytes to the spleen, resulting in a secondary genotoxic effect.

Less genotoxicity information is available for N-methylaniline than for aniline, but the results for the available comparable studies were similar for the two compounds. N-Methylaniline was not mutagenic to *Salmonella typhimurium* strains TA97, TA98, TA100, TA 1535 and TA 1537 or to *Escherichia coli* strain WP2uvrA with or without metabolic activation (Zeiger et al., 1988; GINC, 2003; Hatano Research Institute, undated a). Similarly, aniline was not mutagenic in bacteria under standard test conditions (U.S. EPA, 1992, 2003a; Health Canada, 1994). Both compounds yielded negative results for DNA repair in primary cultured rat hepatocytes when tested at 10^{-6} to 10^{-3} M (Yoshimi et al., 1988).

N-Methylaniline induced chromosomal aberrations in cultured Chinese hamster lung (CHL/IU) cells after 6 hours of exposure with metabolic activation or 24 hours of exposure without metabolic activation (GINC, 2003; Hatano Research Institute, undated b). Aniline did not induce chromosomal aberrations in a different strain of Chinese hamster lung (Don) cells (U.S. EPA, 1992). In other studies, aniline tested positive for genotoxic effects, increasing the frequency of sister chromatid exchanges in human and hamster cells, increasing DNA damage in cultured mouse lymphoma cells, and transforming mouse Balb/3T3 cells (U.S. EPA, 1992). In *in vivo* studies, aniline increased the frequencies of micronucleus formation in bone marrow of rats and mice, and sister chromatid exchanges in bone marrow of mice, but not rats (U.S. EPA, 1992). Because of these uncertainties in mode of action and lack of adequate toxicokinetic information, a non-genotoxic mode of action may not be appropriate for N-methylaniline.

PROVISIONAL WEIGHT-OF-EVIDENCE CLASSIFICATION

No human data are available for the carcinogenicity of N-methylaniline and the animal data are not adequate because the study designs do not meet current standards (Sander, 1971; Greenblatt et al., 1971): group sizes were too small, exposure durations were insufficient, too few dose levels were administered, and the range of tissues evaluated for histopathology was limited. Under the current guidelines (U.S. EPA, 2005b), there is *inadequate information to assess the carcinogenic potential* of N-methylaniline.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Since no chronic inhalation data are available for calculating a quantitative estimate of carcinogenic risk from inhalation exposure to aniline, no provisional estimate of carcinogenic risk can be derived for inhalation exposure to N-methylaniline. Lack of oral carcinogenic data and pharmacokinetic/pharmacodynamic information also precludes derivation of a provisional oral slope factor for N-methylaniline.

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