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Provisional Peer Reviewed Toxicity Values for

2,4-Dimethylphenol
(CASRN 105-67-9)

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 2,4-DIMETHYLPHENOL (CASRN 105-67-9)

Background

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

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

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

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

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

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

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

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and

circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

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

Questions Regarding PPRTVs

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

INTRODUCTION

2,4-Dimethylphenol (2,4-DMP), also known as m-xylenol, 2,4-xylenol or m-4-xylenol, is a naturally occurring, substituted phenol derived from the cresol fraction of petroleum or coal tars. 2,4-DMP has the empirical formula $C_8H_{10}O$ (Figure 1). It is used in the manufacture of a wide range of commercial products for industry and agriculture. There are six isomeric forms of dimethylphenol that exist (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethylphenol).

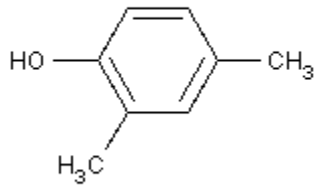

Figure 1. 2,4-Dimethylphenol Structure

The EPA's Integrated Risk Information System (IRIS) (U.S. EPA, 2007) lists a chronic oral reference dose (RfD) of 2E-2 mg/kg-day for 2,4-dimethylphenol based upon data in an unpublished 90-day gavage study in mice (U.S. EPA, 1989). The chronic RfD was derived from

the NOAEL of 50 mg/kg-day for clinical signs of toxicity (lethargy, prostration and ataxia) and a composite uncertainty factor of 3000 (10 for interspecies variability, 10 for intraspecies variability and 30 for lack of chronic toxicity data, data in a second species and reproductive/developmental studies). IRIS does not list a chronic inhalation reference concentration (RfC) or derive a cancer oral slope factor or inhalation unit risk for 2,4-DMP (U.S. EPA, 2007). The HEAST (U.S. EPA, 1997) lists a subchronic RfD of 0.2 mg/kg-day based on the NOAEL from the same principal study identified by IRIS and an uncertainty factor of 300. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not include an RfD or carcinogenicity assessment for 2,4-dimethylphenol. An Ambient Water Quality Criteria Document for 2,4-DMP does not include an RfD or carcinogenicity assessment, but does lists a criterion level of 400 μg/L based upon undesirable organoleptic qualities, which is more a function of aesthetic property of water than a health effect (U.S. EPA, 1980). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes both a Health and Environmental Effects Profile (HEEP) (U.S. EPA, 1986) and a Health Effects Assessment (HEA) (U.S. EPA, 1985) for dimethylphenols. Neither the HEEP (U.S. EPA, 1986) nor the HEA (U.S. EPA, 1985) derived toxicity values for 2,4-DMP, citing insufficient data. Neither the ATSDR (2006), National Toxicology Program (NTP) (2006), International Agency for Research on Cancer (IARC) (2006) nor the World Health Organization (WHO) (2006) has produced documents regarding 2,4-DMP. No occupational exposure limits have been derived by the Occupational Safety and Health Administration (OSHA), the National Institute of Occupational Safety and Health (NIOSH), or the American Conference of Governmental Industrial Hygienists (ACGIH).

Literature searches for studies relevant to the derivation of provisional toxicity values for 2,4,-DMP (CASRN 105-67-9) were conducted from 1965 to August 2006 in TOXLINE (supplemented with BIOSIS and NTIS updates), MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX, CANCERLIT and Current Contents.

REVIEW OF PERTINENT LITERATURE

Human Studies

No studies investigating the effects of subchronic or chronic oral or inhalation exposure to 2,4-DMP in humans were identified.

Animal Studies

Oral Exposure

Chronic Studies – No studies investigating the effects of chronic oral exposure to 2,4-DMP in animals were identified.

Subchronic Studies – Studies on the subchronic toxicity of oral exposure to 2,4-dimethylphenol have been conducted in rats exposed for 10 and 90 days (Daniel et al., 1993) and

mice exposed for 14 (U.S. EPA, 1987) and 90 days (U.S. EPA, 1989). Additional studies evaluating the effects of subchronic exposure of animals to oral 2,4-DMP were not identified.

Groups of 10 male and 10 female Sprague-Dawley rats (80 days old) were administered 0 (vehicle control), 60, 120, 600 or 1200 mg/kg body weight of 2,4,-DMP in corn oil by gavage once daily for 10 consecutive days (Daniel et al., 1993). Rats were observed daily for mortality and physiological and behavioral signs of toxicity. Body weights were recorded on days 0, 4 and 6 of treatment and at the end of the study. Blood samples taken at the end of the treatment period were analyzed for the following: white blood cell (WBC) count, red blood cell counts (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), glucose, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, lactate dehydrogenase (LDH) and calcium (Ca^{++}). Organ weights were recorded and gross examination of comprehensive tissues was performed at the end of the treatment period. All tissues from the control and 600 mg/kg groups were examined microscopically. As target organs for 2,4,-DMP were identified, target tissues from the 60 and 120 mg/kg groups were examined microscopically.

All male and female rats treated with 1200 mg/kg body weight 2,4-DMP died prior to completion of the 10-day treatment period (time of death not reported) (Daniel et al., 1993). The study authors attributed the cause of death to 2,4-DMP-induced severe stomach lesions. Mortalities in other groups were as follows: 1 male in the control group, 1 female in the 120 mg/kg group, and 2 females and 1 male in the 600 mg/kg group. The cause of death or relationship to treatment was not reported. No mortalities occurred in the 60 mg/kg group. Clinical and behavioral signs of toxicity were not reported for any dose group. In surviving animals, food and water intake, final body weight and body weight gain were not significantly different from controls in any 2,4-DMP group. Relative liver weight was significantly increased in females, but not males, in the 600 mg/kg group compared to control (Table 1). No increase in relative liver weight was observed in males or females in other 2,4-DMP groups. Relative weights of other organs were not affected by treatment. Absolute organ weights were not reported.

Effects on hematological and clinical chemistry parameters were observed only in the high-dose group, except for decreased AST in females in the 60 mg/kg-day group and decreased Ca^{++} in males in the 120 mg/kg-day group, as shown in Table 2 (Daniel et al., 1993). In general, relative to control, the observed effects were minimal. Significant increases in WBC and Hgb values were observed in females, but not males, in the 600 mg/kg-day treatment group. No other effects on hematological parameters were observed for any treatment group for females or males. In females, mean serum glucose and cholesterol levels were significantly increased in the 600 mg/kg group and AST levels were significantly decreased in the 60 and 600 mg/kg dose groups (Table 2); however, AST levels were not significantly different from control in the 120 mg/kg group. In male rats, serum Ca^{++} was decreased in the 120 and 600 mg/kg groups and AST was significantly decreased in the 600 mg/kg group (Table 2). Serum cholesterol was increased in both males and females treated with 600 mg/kg-day (Table 2).

Table 1. Effect of Oral Treatment of Rats with 2,4-DMP (10 Day Exposure) on Final Body Weight and Relative Liver Weight (Daniel et al., 1993)

Parameter	Treatment Group (mg/kg-day)			
	0	60	120	600
Females				
Number of animals	10	10	9	8
Final body weight (g)	234.2±10.1[a]	237.8±13.0 (101.5)	233.8±11.2 (99.8)	224.9±12.9 (98.0)
Relative Liver weight (%)	2.97±0.24	3.13±0.23 (105.0)	3.19±0.16 (107.0)	3.50±0.32 (117.4)[b]
Males				
Number of animals	9	10	10	9
Final body weight (g)	354.0±19.8	347.9±18.1 (98.3)	358.1±30.2 (101.2)	335.2±21.2 (94.7)
Relative Liver weight (%)	3.05±0.21	3.09±0.22 (101.3)	3.14±0.21 (103.0)	3.29±0.49 (107.9)

[a] Values are means ± Standard Deviation (SD); ( ) = percent of control
[b] Significantly different from control ($p \leq 0.05$), Analysis of Variance (ANOVA)

Table 2. Effect of Oral Treatment of Rats with 2,4-DMP (10 Day Exposure) on Hematology and Serum Chemistry Values (Daniel et al., 1993)

Parameter	Treatment Group (mg/kg-day)			
	0	60	120	600
Females				
WCB ($x10^3$)	7.0±1.8[a]	8.6±1.7 (122.8)	7.3±1.8 (104.3)	9.5±2.3 (135.7)[b]
Hgb (g/dL)	14.9±0.9	15.1±0.5 (101.3)	15.3±0.7 (102.7)	16.2±1.2 (108.7)[b]
Glucose (mg/dL)	95.5±15.4	117.4±14.1 (122.9)	107.8±22.1 (112.9)	138.0±24.2 (144.5)[b]
Cholesterol (mg/dL)	71.6±12.1	78.6±11.6 (109.8)	86.5±9.7 (120.8)	110.9±38.0 (154.9)[b]
AST (IU^c/L)	111.3±18.7	89.6±13.1 (80.5)[b]	95.3±20.5 (86.5)	84.4±13.4 (75.8)[b]
Ca^{++} (mg/dL)	10.1±0.5	10.2±0.6 (101.0)	10.5±0.5 (104.0)	10.7±1.2 (103.0)
Males				
WCB ($x10^3$)	8.6±1.3	10.5±4.0 (122.1)	9.5±1.6 (110.5)	11.5±0.3 (133.7)
Hgb (g/dL)	15.6±0.5	15.8±0.6 (101.3)	16.0±0.7 (102.6)	16.1±0.4 (103.2)
Glucose (mg/dL)	109.2±12.3	120.4±25.9 (110.3)	124.3±27.9 (113.8)	135.9±27.6 (124.5)
Cholesterol (mg/dL)	62.1±10.6	64.2±8.8 (103.4)	60.3±9.8 (97.1)	65.0±17.0 (104.7)[b]
AST (IU/L)	102.5±18.7	101.1±38.2 (98.6)	99.8±15.3 (97.4)	80.4±13.1 (78.4)[b]
Ca^{++} (mg/dL)	10.6±0.3	10.1±0.3 (95.2)	9.7±0.6 (91.5)[b]	9.5±1.0 (89.6)[b]

[a] Values are means ± SD; ( ) = percent of control
[b] Significantly different from control ($p \leq 0.05$), ANOVA
[c] International Units (IU)

Based on the cause of death (severe stomach lesions) for all rats in the 1200 mg/kg group, the stomach was identified as the primary target organ for exposure to 2,4-DMP by gavage (Daniel et al., 1993). In the 600 mg/kg group, lesions of the forestomach (including epithelial hypertrophy, hyperkeratosis and mucosal vacuolar degeneration) were observed in male and female rats. Although the authors state that the incidence and severity of forestomach lesions increased with dose, specific dose-response data were not presented and no information on stomach lesions in the 60 and 120 mg/kg group was reported. Therefore, it is unclear from this report if forestomach lesions were observed in all 2,4-DMP dose groups. Thus, due to inadequacy of reporting, NOAEL and LOAEL values cannot be determined for this 10-day study.

Groups of 10 male and 10 female Sprague-Dawley rats (80 days old) were administered 0 (vehicle control), 60, 180 or 540 mg/kg body weight of 2,4-DMP in corn oil once daily for 90 consecutive days by gavage (Daniel et al., 1993). Rats were observed daily for mortality and clinical and behavioral signs of toxicity. Body weights and food and water consumption were recorded weekly. Blood samples taken at the end of the treatment period were analyzed for the following: WBC count, red blood cell counts (RBC), platelet count, hemoglobin (Hgb), Hct, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), glucose, BUN, creatinine, ALP, AST, ALT, cholesterol, triglycerides, LDH, gamma glutamyl transpeptidase (GGT), total bilirubin, direct bilirubin, total protein, albumin (A), globulin (G), Ca^{++}, sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), phosphate (PO_4) and magnesium (Mg^{++}). At the end of the treatment period, gross pathological examination was conducted on rats from all treatment groups. The stomach of all surviving animals was examined microscopically and histopathological examination was performed on all tissues from the control and high-dose group (180 mg/kg).

All females and 6 of 10 males treated in the 540 mg/kg group died by the fifth day of treatment (Daniel et al., 1993). Subsequently, 6 females and 6 males were added to the 540 mg/kg group, with a total of 3/16 females and 7/16 males surviving for the 90-day treatment period. The cause of death for all animals in the 540 mg/kg group was reported as corrosive effects of 2,4-DMP on the esophagus and stomach, based on findings of the gross pathological examination. No mortalities or clinical signs of toxicity were observed in female or male rats in control or other 2,4-DMP treatment groups. Final body weight was decreased approximately 10% in females in the 180 and 540 mg/kg groups (statistically significant only in the 180 mg/kg-day group) and in males in the 540 mg/kg group. Changes in relative organ weights generally appeared to be secondary to changes in body weight, with small increases in relative brain, liver, kidney, and (in males) testes weights at doses that also produced decreases in body weight (Table 3). Relative thymus weight was significantly decreased in males at 60 mg/kg-day, but not in higher dose males or females.

Hematological analysis revealed a 2.4% increase ($p \leq 0.05$) in MCV in females treated with 540 mg/kg body weight 2,4-DMP compared to control; however, the authors state that the magnitude of change was not considered biologically significant (Daniel et al., 1993). No other

Table 3. Effect of Oral Treatment of Rats with 2,4-DMP (90 Day Exposure) on Final Body Weight and Relative Organ Weights (Daniel et al., 1993)

Parameter	Treatment Group (mg/kg-day)			
	0	60	180	540
Females				
Number of animals	10	10	10	3
Final body weight (g)	269.0±26.2[a]	263.1±21.5 (97.8)	240.3±24.4 (89.3)[b]	244.6±21.0 (90.9)
Relative brain weight (%)	0.81±0.06	0.81±0.08 (100)	0.88±0.07 (108.6)[b]	0.93±0.13 (114.8)
Relative kidney weight (%)	0.74±0.04	0.70±0.04 (94.6)[b]	0.80±0.08 (108.1)[b]	0.82±0.04 (110.8)
Relative liver weight (%)	2.90±0.16	2.84±0.31 (97.9)	3.13±0.32 (107.9)	3.21±0.13 (110.7)[b]
Relative thymus weight (%)	0.12±0.03	0.12±0.02 (100)	0.10±0.02 (83.3)	0.11±0.03 (91.7)
Males				
Number of animals	10	10	10	7
Final body weight (g)	492.8±40.2	516.6±56.4 (104.8)	507.3±21.2 (102.9)	442.0±41.0 (89.7)[b]
Relative brain weight (%)	0.48±0.04	0.46±0.05 (95.8)	0.46±0.03 (95.8)	0.52±0.03 (108.3)[b]
Relative kidney weight (%)	0.70±0.04	0.67±0.04 (95.7)	0.69±0.04 (98.6)	0.77±0.06 (110.0)[b]
Relative liver weight (%)	3.17±0.39	2.97±0.32 (93.7)	3.13±0.18 (98.7)	3.30±0.35 (104.1)
Relative thymus weight (%)	0.08±0.01	0.06±0.01 (75.0)[b]	0.07±0.01 (87.5)	0.07±0.02 (87.5)
Relative testes weight (%)	0.72±0.12	0.66±0.09 (91.7)	0.68±0.07 (94.4)	0.83±0.08 (115.3)[b]

[a] Values are means ± SD; ( ) = percent of control
[b] Significantly different from control ($p \leq 0.05$), ANOVA

hematology parameters were affected by 2,4-DMP treatment. Effects on clinical chemistry parameters were minor (Table 4). In females, mean phosphate levels in the low dose group and AST levels in the middle-dose group were significantly decreased. In high-dose females, the BUN/creatinine ratio and cholesterol levels were increased and creatinine and chloride levels were decreased. In high-dose males, serum creatinine and AST were significantly decreased, whereas cholesterol, triglycerides and Mg^{++} were significantly increased.

Gross pathological examination at the end of the treatment period revealed a small, red thymus in a small percentage (percentage not reported) of males in the 60 and 540 mg/kg groups, but not in the 180 mg/kg group. Incidence data for gross thymus lesions were not reported and histopathological examination of the thymus was not performed. At the end of the 90-day treatment period, histopathological examination of the forestomach showed hyperkeratosis and epithelial hyperplasia in all males in the 180 and 540 mg/kg groups, 60% of females in the 180 mg/kg group and all females in the 540 mg/kg group. Severity of lesions increased with dose (data on severity not reported). Although histopathological assessment of the stomach was performed in the low-dose group, no data or information were presented; thus, it is unclear if lesions were present in rats treated with 60 mg/kg-day 2,4-DMP. No other treatment-related histopathological changes were observed in male or female rats. The study authors identified 60 mg/kg body weight as the NOAEL since "no biologically significant changes in frequency or severity of adverse effects" relative to control were observed; however, the "biologically

Table 4. Effect of Oral Treatment of Rats with 2,4-DMP (90 Day Exposure) on Serum Chemistry Values (Daniel et al., 1993)

Parameter	Treatment Group (mg/kg-day)			
	0	60	180	540
Females				
Creatinine (mg/dL)	0.71±2.8[a]	0.68±0.11 (95.8)	0.70±0.13 (98.6)	0.57±0.06 (80.3)[b]
BUN/Creatinine ratio	26±5	26±4 (100)	31±7 (119.2)	41±5 (157.7)[b]
AST (IU/L)	149±29	137±69 (91.9)	121±15 (81.2)[b]	169±11 (113.4)
Cholesterol (mg/dL)	35±7	42±7 (120)	43±9 (122.9)	73±14 (208.6)[b]
Triglycerides (mg/dL)	64±17	65±20 (101.6)	56±27 (87.5)	63±5 (98.4)
Cl^- (mEq/L)	98±4	100±2 (102.0)	99±3 (101.0)	95±2 (96.9)[b]
Mg^{++} (mEq/L)	2.1±0.2	2.0±0.1 (95.2)	2.1±0.1 (100)	2.2±0.2 (104.8)
PO_4 (mEq/L)	5.3±0.5	4.5±0.9 (84.9)[b]	5.4±1.0 (101.9)	6.6±0.8 (124.5)
Males				
Creatinine (mg/dL)	0.61±0.06	0.61±0.11 (100)	0.59±0.03 (96.7)	0.54±0.05 (88.5)[b]
BUN/Creatinine ratio	30±6	32±7 (103.2)	30±4 (96.8)	34±5 (109.7)
AST (IU/L)	115±15	165±93 (143.5)	113±26 (98.3)	99±6 (86.1)[b]
Cholesterol (mg/dL)	38±9	40±13 (105.3)	43±12 (113.2)	48±5 (126.3)[b]
Triglycerides (mg/dL)[c]	38±9	40±13 (105.3)	43±12 (113.2)	48±5 (126.3)[b]
Cl^- (mEq/L)	100±1	101±2 (101)	100±1 (100)	100±2 (100)
Mg^{++} (mEq/L)	1.8±0.2	1.9±0.1 (105.6)	1.9±0.2 (105.6)	2.0±0.1 (111.1)[b]

[a] Values are means ± SD; ( ) = percent of control
[b] Significantly different from control ($p \leq 0.05$), ANOVA
[c] For male rats, values for triglycerides as reported by study authors, were identical to those for cholesterol. Comparison of triglyceride and cholesterol concentrations for males and females indicated that triglyceride concentrations for males were incorrectly reported by Daniel et al. (1993).

significant" effects serving as the basis for the LOAEL of 180 mg/kg body weight-day were not specifically identified. Based on results of this study, the stomach appears to be a target organ for orally administered 2,4,-DMP. Due to ambiguous reporting, it is unclear if 2,4-DMP induced stomach lesions in the 60 mg/kg-day group, introducing significant uncertainty to the NOAEL and LOAEL values reported by the study authors.

The oral toxicity of 2,4-DMP was evaluated in a 90-day study in albino mice (U.S. EPA, 1989). Data from this unpublished study serve as the basis for the chronic RfD for 2,4-DMP listed by IRIS (U.S. EPA, 2007). Groups of 30 male and 30 female albino mice [strain Crl:CD-1(ICR)BR-VAF+] were administered 5, 50 or 250 mg/kg body weight 2,4-DMP in corn oil by gavage for 90 days. Untreated control and vehicle control, consisting of 30 male and 30 female mice per group, were included. Mice were observed twice daily throughout the treatment period for mortality, morbidity and signs of toxicity. A 30-day interim sacrifice was performed on eight

males and nine females from each group. Body weights and food consumption were recorded weekly. Blood was analyzed for hematological (Hgb, Hct, RBC, total and differential leukocyte count, platelet count, reticulocyte count, MCV, MCH and MCHC) and clinical chemistry parameters (Ca^{++}, Cl^{-}, PO_4, K^{+}, Na^{+}, glucose, creatinine, BUN, ALT, AST, LDH, ALP, albumin, globulin, total protein, total bilirubin and cholesterol) at the interim sacrifice (for mice sacrificed at 30 days) and at the end of the 90-day exposure period. Ophthalmologic examinations were conducted prior to study initiation and in all surviving mice at study termination. Necropsy was performed on all animals found dead during the study and in all surviving animals at the end of the 90-day exposure period. Histopathological examination of comprehensive tissues was performed at the end of the treatment period and in all animals dying prior to study completion.

A total of 15 animals (0 in untreated control, 3 in vehicle control, 4 in 5 mg/kg, 3 in 50 mg/kg and 5 in 250 mg/kg groups) died during the treatment period; deaths were attributed to technical errors (ruptured esophagus) and not considered as treatment-related by study authors (U.S. EPA, 1989). Body weight and food consumption were similar to controls for all 2,4-DMP groups. Clinical signs of toxicity were not observed during the first 6 weeks of treatment. From week 7 through the end of the treatment period, squinting, lethargy, prostration and ataxia were observed in high-dose males and females following daily dosing. No treatment-related ophthalmologic findings were observed in any 2,4-DMP group.

At the interim sacrifice, small decreases in MCV (4.3% decrease, $p \leq 0.05$) and MCH (3.7% decrease, $p \leq 0.05$) were observed for females in the high-dose group compared to vehicle control, while larger decreases were observed in BUN levels for females in the mid- (32.5% decrease, $p \leq 0.05$) and high-dose (21.7% decrease, $p \leq 0.05$) groups (U.S. EPA, 1989). A significant increase in cholesterol levels (79% increase, $p \leq 0.05$) was observed for males in the low-dose group. No effects on other hematological or clinical chemistry parameters were observed at the interim sacrifice. At the end of the 90-day treatment period, all hematology parameters in 2,4-DMP treated mice were similar to control. Changes in clinical chemistry and organ weights observed after 90 days of treatment were sporadic, with no dose-related patterns of change. The organ weight data are shown in Table 5. No treatment-related gross pathological or histological findings, including lesions of the stomach, were observed. Based on clinical signs of toxicity in the high-dose 2,4-DMP group, NOAEL and LOAEL values were identified as 50 and 250 mg/kg-day.

According to IRIS (U.S. EPA, 2007), an unpublished 14-day gavage study with 2,4-DMP (U.S. EPA, 1987) was conducted by the same laboratory as the 90-day gavage study in mice (U.S. EPA, 1989). Results of the 14-day study revealed signs of toxicity (lethargy, prostration and ataxia) in males and females exposed to 250 mg/kg-day, the same dose at which signs of toxicity effects were observed in the 90-day study. No additional information pertaining to this study was provided by IRIS (U.S. EPA, 2007). This study was not available for review.

Inhalation Exposure

No studies investigating the effects of subchronic or chronic inhalation exposure to 2,4-DMP in animals were identified.

Table 5. Effect of Oral Treatment of Mice with 2,4-DMP (90-Day Exposure) on Final Body Weight and Relative Organ Weights (U.S. EPA, 1989)

Parameter	Treatment Group (mg/kg-day)				
	Control	Vehicle Control	5	50	250
Females					
Body weight (g)	27.2±3.9[a]	26.6±2.4	26.4±2.8	26.6±3.1	26.1±1.6
Liver weight (g)	1.1967	1.1001	1.1136	1.1285	1.1425
Relative liver weight(g/100 g)	4.4289	4.1072	4.1953	4.2614	4.3733
Spleen weight (g)	0.0959	0.0846	0.0854	0.0796	0.0898
Relative spleen weight (g/kg)	0.3500	0.1607	0.1639	0.1518	0.1703
Adrenal weight (g)	0.0124	0.0108	0.0142[b]	0.0115	0.0110
Relative adrenal weight (g/100 g)	0.0467	0.0403	0.0548[b]	0.0437	0.0424
Males					
Body weight (g)	33.9±3.3	32.5±2.9	33.3±2.7	32.5±3.3	31.6±3.5
Liver weight (g)	1.4430	1.2658	1.3412	1.2985	1.3166[b]
Relative liver weight(g/100 g)	4.2744	3.9064	4.0421	3.9955	4.1695[b]
Spleen weight (g)	0.1081	0.0758	0.0907[b]	0.0742	0.0747
Relative spleen weight (g/kg)	0.3272	0.2337	0.2740	0.2282	0.2368
Adrenal weight (g)	0.0093	0.0099	0.0108	0.0083	0.0082
Relative adrenal weight (g/100 g)	0.0275	0.0303	0.0324	0.0256	0.0263

[a] Values are means ± SD, or means only
[b] Significantly different from vehicle control ($p \leq 0.05$)

Other Studies

Dermal- The immunomodulatory effects of 2,4-DMP were examined in six- to eight-week old male BALB/cA mice following short-term (3-day) dermal exposure (Yamano et al., 2007). Groups of mice (n = 3/group) were exposed to 25 µL of 1M 2,4-DMP (equivalent to 100 mg/kg) or vehicle (acetone/olive oil, 4:1) through application to the dorsum of both ears for 3 consecutive days. Three or five days after the last exposure to 2,4-DMP, auricular lymph nodes (LN) were excised from each mouse and prepared for evaluation using the murine local lymph node assay (LLNA), or were processed for primary cell culture and subsequent cytokine profiling, respectively. The LLNA allows for determination of whether a chemical acts as an immediate or delayed type immunogen which is related to the relative proportions of or balance between type-1 and type-2 T helper (Th-1 and Th-2, respectively) cells. Th-1 and Th-2 cells are differentiated by the types of cytokines produced in response to an immunogen. Th-1 cells secrete pro-inflammatory cytokines such as interferon-γ (IFN-γ), whereas Th-2 cells secrete anti-inflammatory cytokines such as interleukin-4 (IL-4). Thus, these two subsets of T helper cells are in essence functional antagonists of one another. In addition to the LLNA, primary splenocyte cultures from immunologically naïve mice were used for *in vitro* analysis of cell viability and cytokine profiling following 48 hr. of 2,4-DMP exposure. LLNA data suggested

that 2,4-DMP caused lymph node proliferation by acting as an immunogen via the dermal route of exposure. However, 2,4-DMP failed to stimulate lymphocyte secretion of the pro-inflammatory cytokine IFN-γ, or inhibit the anti-inflammatory cytokine IL-4 compared to control. Thus, it appears that 2,4-DMP is a weak inducer of a type-1 reaction in T helper cells (i.e. Th-1) following dermal absorption. Specifically, the results suggest that while dermal 2,4-DMP exposure induces an apparent increase in lymphocyte population of auricular nodes, the immunomodulatory effect (i.e. the ability to tip the balance between a Th-1 or Th-2 type immune response) was not significantly different from vehicle treated controls.

Toxicokinetic – Little information is available regarding the toxicokinetics of 2,4-DMP. In general, dimethylphenol isomers undergo extensive absorption from the gastrointestinal tract (Miyamoto et al., 1969). Results of a kinetic study in male Sprague-Dawley rats indicate that intravenously administered 2,4-DMP undergoes rapid distribution, with accumulation in the brain, liver and fat (Kaka et al., 1982). Metabolism to glucuronide and sulfate conjugates was rapid and nearly complete within 30 minutes of administration (Kaka et al., 1982).

Genotoxicity – All available evidence indicates that 2,4-DMP, like the other dimethylphenol isomers, is not genotoxic. All dimethylphenol isomers tested negative in reverse mutation assays with *Salmonella tymphimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without activation (Pool and Lin, 1982; Florin et al., 1980; Mortelmans et al., 1986). In a reverse mutation assay with *Escherichia coli* strain Sd 4-72, 2,4-DMP tested negative (Szybalski et al., 1958). 2,4-DMP also tested negative in a sister-chromatid exchange assay in isolated human lymphocytes (Jannson et al., 1986).

Tumor Promoting Activity – Although no subchronic or chronic oral or inhalation carcinogenicity studies have been performed on dimethylphenol isomers, data are available to suggest that the 2,4-, 2,5-, 3,4- and 3,5-DMP isomers exhibit tumor promoting activity on mouse skin (Boutwell and Bosch, 1959). All isomers except 2,6-DMP produced a small increase in carcinoma incidence when applied to skin without an initiation. However, the available data are not sufficient to assess the carcinogenicity of 2,4-DMP or other dimethylphenol isomers.

DERIVATION OF A PROVISIONAL SUBCHRONIC ORAL RfD FOR 2,4-DIMETHYLPHENOL

No studies on the effects of oral exposure to 2,4-DMP in humans are available. Subchronic toxicity studies in rats and mice identify the stomach and thymus as possible target organs for oral 2,4-DMP. The 10-day oral toxicity study in rats showed dose-related irritant and corrosive effects of the esophagus and forestomach following administration of 2,4-DMP by gavage (Daniel et al., 1993). Although the study report clearly indicates that histopathological changes to the forestomach were observed in rats treated with 600 and 1200 mg/kg-day, due to inadequate reporting, it is unclear if effects on the forestomach were present at lower doses (60 and 120 mg/kg-day). Irritant and corrosive effects of the esophagus and forestomach were observed in rats exposed to 180 and 540 mg/kg-day for 90 days; however, results of histopathological examination of the forestomach in the 60 mg/kg-day were not reported. Although 2,4-DMP clearly produces adverse effects to the esophagus and forestomach of rats

following administration by gavage, the available dose-response information is not adequate for the basis of the subchronic p-RfD.

Decrease in relative thymus weight was observed in male, but not female, rats treated with 60 mg/kg-day for 90 days, although the magnitude of change was small (Daniel et al., 1993). Gross pathological examination revealed a small, red thymus in a "small percentage" of surviving males in the 60 and 540 mg/kg groups, but not in the 180 mg/kg group. Thus, a clear dose-response relationship was not observed. No information was reported on histopathological examination of the thymus. Since thymus and immune system function of rats was not assessed, the biological significance of decreased thymus weight and small, red thymus is uncertain. Therefore, the effect of 2,4-DMP on thymus weight was not selected as the basis for the subchronic p-RfD.

The 90-day gavage study in mice reported general signs of toxicity, including squinting, lethargy, prostration and ataxia in males and females following daily dosing with 250 mg/kg-day, establishing a NOAEL of 50 mg/kg-day (U.S. EPA, 1989). Although signs of clinical toxicity are not very sensitive endpoints, comprehensive toxicological endpoints were examined, including histopathology, and the study was well-reported. Thus, the NOAEL of 50 mg/kg-day for signs of toxicity was selected as the basis of the subchronic p-RfD. As indicated in the Introduction section of this document, this is the same study and critical effect used to derive the chronic RfD for 2,4-DMP listed by IRIS (U.S. EPA, 2007).

The **subchronic p-RfD of 5E-2 mg/kg-day** was derived from the NOAEL of 50 mg/kg-day for signs of clinical toxicity as follows:

$$\begin{aligned} \text{p-RfD} &= \text{NOAEL} \div \text{UF} \\ &= 50 \text{ mg/kg-day} \div 1000 \\ &= 0.05 \text{ mg/kg-day or } 50 \text{ } \mu\text{g/kg-day} \\ &= \textbf{5E-2 mg/kg-day} \end{aligned}$$

The uncertainty factor (UF) of 1000 was composed of the following:

- An UF of 10 was applied for interspecies extrapolation to account for potential pharmacodynamic and pharmacokinetic differences between mice and humans.
- A default 10-fold UF for intraspecies differences was used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans.
- An UF of 10 was included for database insufficiencies due to the lack of oral developmental studies and a multi-generation reproduction study.

Confidence in the principle study is medium, since it examined appropriate and comprehensive endpoints and identified both LOAEL and NOAEL values. The database for oral exposure to 2,4-DMP includes only two subchronic gavage studies conducted in rats and mice, with different effects in each species. Furthermore, the database provides no information on developmental and reproductive studies. Low confidence in the database and the oral subchronic p-RfD results.

FEASIBILITY FOR DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR 2,4-DIMETHYLPHENOL

No studies investigating the effects of subchronic or chronic inhalation exposure to 2,4-DMP in humans or animals were identified. The lack of subchronic and chronic inhalation data precludes derivation of subchronic and chronic p-RfCs for 2,4-DMP.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 2,4-DIMETHYLPHENOL

No studies evaluating the carcinogenic potential of oral or inhalation exposure to 2,4-DMP in humans were identified in the available literature. Cancer bioassays for 2,4-DMP have not been conducted in animals for either oral or inhalation exposure. Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), inadequate information is available to assess the carcinogenic potential of 2,4-DMP.

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