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Provisional Peer-Reviewed Toxicity Values for

Hexamethylphosphoramide
(CASRN 680-31-9)

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Harlal Choudhury, DVM, PhD, DABT
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

ICF International
9300 Lee Highway
Fairfax, VA 22031

PRIMARY INTERNAL REVIEW

Ghazi Dannan, PhD
National Center for Environmental Assessment, Washington, DC

Anuradha Mudipalli, MSc, PhD
National Center for Environmental Assessment, Research Triangle Park, NC

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Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
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
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
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR HEXAMETHYLPHOSPHORAMIDE (CASRN 680-31-9)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents and appropriate use of this PPRTV assessment should 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).

INTRODUCTION

Hexamethylphosphoramide (HMPA), or hexamethylphosphoric triamide, is used as a solvent for polymers, a selective solvent for gases, and a thermal and ultraviolet radiation degradation stabilizer in various polymers (International Agency for Research on Cancer [IARC], 1999). The empirical formula for HMPA is $C_6H_{18}N_3OP$ (see Figure 1). A table of physicochemical properties is provided below (see Table 1). In this document, unless otherwise noted, "statistically significant" denotes a *p*-value of <0.05.

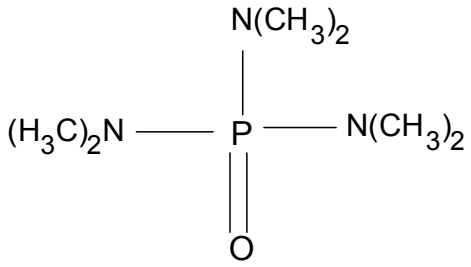

Figure 1. Hexamethylphosphoramide Structure

Table 1. Physicochemical Properties Table
Hexamethylphosphoramide[a] (CASRN 680-31-9)

Property (unit)	Value
Boiling point (ºC)	232.5
Melting point (ºC)	7.2
Density (g/cm^3)	1.03
Vapor pressure (Pa at 25ºC)	4
pH (unitless)	Not available
Solubility in water (mg/L)	1×10^6
Relative vapor density (air = 1)	6.18
Molecular weight (g/mol)	179.2
Flash point (°C)	105
Octanol/water partition coefficient (unitless)	0.28

[a]Values from http:// www.cdc.gov/niosh/ipcsneng/neng0162.html and Chem ID Plus (2011).

The EPA IRIS (U.S. EPA, 2010a) database does not list a chronic oral reference dose (RfD), a chronic inhalation reference concentration (RfC), or a cancer assessment for HMPA. Subchronic or chronic RfDs or RfCs for HMPA are not listed in the HEAST (U.S. EPA, 1997) or on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006); the HEAST cites

inadequate data for quantitative risk assessment. No assessments are reported on the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994) for HMPA. The California Environmental Protection Agency (CalEPA, 2008a, 2009a) has not derived toxicity values for exposure to HMPA.

The American Conference of Governmental Industrial Hygienists (ACGIH, 2004) considers HMPA an A3 substance ("*Confirmed Animal Carcinogen with Unknown Relevance to Humans*") and has assigned a "skin" notation (indicating possible skin absorption) for HMPA. The National Institute of Occupational Safety and Health (NIOSH, 2005) considers HMPA to be a potential occupational carcinogen and recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration. The Occupational Safety and Health Administration (OSHA, 2010) has not derived any occupational exposure limits for HMPA.

Neither the World Health Organization (WHO, 2010) nor the Agency for Toxic Substances and Disease Registry (ATSDR, 2010) has published a toxicological review on HMPA. The IARC (1999) has classified HMPA as "*Possibly Carcinogenic to Humans"* (Group 2B) based on evidence from a 2-year inhalation study in rats. The *11th Report on Carcinogens* (National Toxicology Program [NTP], 2005) lists HMPA as "*Reasonably Anticipated to be a Human Carcinogen*" based on sufficient evidence of carcinogenicity in experimental animals. CalEPA lists HMPA as a carcinogen (CalEPA, 2008b). CalEPA (2009b,c) has not prepared a quantitative estimate of the carcinogenic potential for HMPA.

Literature searches were conducted on sources published from 1900 through November 2010, for studies relevant to the derivation of provisional toxicity values for HMPA, CAS No. 680-31-9. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for toxicity values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides information for all of the potentially relevant studies. Entries for the principal studies are bolded.

Table 2. Summary of Potentially Relevant Data for Hexamethylphosphoramide (CASRN 680-31-9)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Human								
			1. Oral (mg/kg-day)[a]					
None								
			2. Inhalation (mg/m^3)[a]					
None								
Animal								
			1. Oral (mg/kg-day)[a]					
Subchronic	**10/10, Charles River CD rat, drinking water, 7 days (d)/week (wk), 90 d**	**0, 1.2, 15, 42, 123 (m); 0, 2.3, 20, 63, 229 (f)**	**Dose-related increase in nasal and tracheal lesions, significantly increased in severity at 15 mg/kg-day (males) and 20 mg/kg-day (females) Testicular atrophy and significantly reduced body weight and absolute and relative testes weights in 123 mg/kg-day males**	**1.2**	**Not run**	**15**	**Keller et al. (1997)**	**PS**
	10 male, Charles River CD rat, gavage, 7 d/wk, 90 d (Note, an additional 10 male Charles River CD rats received 40 mg/kg-day by osmotic minipumps, implanted subcutaneously)	0, 15, 40, 120	Increased nasal lesions at all doses Testicular atrophy and significantly reduced body weight in 120-mg/kg-day males (Nasal lesions at 40 mg/kg-day in implant study)	None	Not run	15	Keller et al. (1997)	
	20 male, Sherman rat, dietary, 52−72 d	0, 106−127	Increase in lung lesions and lung weight	None	Not run	None	Kimbrough and Sedlak (1968)	

Table 2. Summary of Potentially Relevant Data for Hexamethylphosphoramide (CASRN 680-31-9)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
	20/20, Albino rat, gavage, 7 d/wk, 92 d	0, 2, 10, 50	Decrease in thyroid weight in females at 10 mg/kg-day Increase in respiratory disease, decrease in growth rate, and decrease in liver, kidney, and thyroid weights at 50 mg/kg-day	2	Not run	10	Shott et al. (1971)	
Chronic	15 male, Sherman rat, dietary, 2 yr	0, 0.78, 1.56, 3.12, 6.25	Increase in lung disease at all doses	None	Not run	None	Kimbrough and Gaines (1973)	
Developmental	10 female at 200 mg/kg-day, 8 female at 0 mg/kg-day, Sherman rat, gavage, 7 d before mating to GD 20	0, 200	No abnormalities; weight of fetus and placenta, number of rats per litter, and number of resorption sites similar to controls	None	Not run	None	Kimbrough and Gaines (1966)	
Reproductive	40/40, Albino rat, gavage, 7 d/wk, 2-generation, 169 d	0, 2, 10	No teratogenicity Pneumonia in P1 rats at 2 and 10 mg/kg-day noted as cause of reduction in fertility index at these doses	10	Not run	None	Shott et al. (1971)	
	Sherman rat (number not provided males), gavage, 56 d	25	No effect on fertility or on the testes	None	Not run	None	Kimbrough and Gaines (1966)	
	5−9 male, Sherman rat, gavage, once	0, 500, 1000, 2000	Partial or complete testicular atrophy at 1000 and 2000 mg/kg-day Pneumonia in rats at all doses	None	Not run	None	Kimbrough and Gaines (1966)	
	4−10 male, Sherman rat, gavage, 36−99 d	0, 100, 200, 400	Partial or complete testicular trophy at 100, 200, and 400 mg/kg-day Pneumonia in rats at all doses	None	Not run	None	Kimbrough and Gaines (1966)	
	3−11 male, Sherman rat, dietary, 61−103 d	0, 40−80	Partial or complete testicular atrophy and pneumonia at all doses	None	Not run	None	Kimbrough and Gaines (1966)	

Table 2. Summary of Potentially Relevant Data for Hexamethylphosphoramide (CASRN 680-31-9)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
	6 female, Sherman rat, (route not provided), once	0, 2500	No effects on female reproductive organs	None	Not run	None	Kimbrough and Gaines (1966)	
	4−8 male, Wistar rat, by mouth, daily, 3 wk	25, 50, 100	Decrease in litter size at 50 and 100 mg/kg-day Decrease in testes weight and damage to the testes at 100 mg/kg-day	None	Not run	None	Jackson et al. (1969)	
	8 male, Wistar rat, by mouth, daily, 6 d	500 (different purity)	Decrease in litter size, damage to the testes	None	Not run	None	Jackson et al. (1969)	
	15 male, Sherman rat, dietary, once	0, 6.25	No effect on reproduction	None	Not run	None	Kimbrough and Gaines (1973)	
	Rat, mouse, rabbit (number and strain unknown, male), by mouth, 5−21 doses/d	100, 250, 500 (rat) 500 (mouse) 100 (rabbit)	Reduced weekly litter size and reduced sperm count	None	Not run	None	Jackson and Craig (1966)	
Carcinogenic	None							
2. Inhalation (mg/m^3)[a]								
Subchronic	None							
Chronic	None							
Developmental	None							
Reproductive	None							
Carcinogenic	120/120, Charles River Caesarean or Charles-CD Sprague-Dawley-derived rat, 5 d/wk, 3−24 mo	0, 0.293, 0.962, 8.68*	Nasal tumors, rhinitis, degeneration, squamous metaplasia, and dysplasia at all doses	None	Not run	None	Lee and Trochimowicz (1982a,b,c, 1984)	

Table 2. Summary of Potentially Relevant Data for Hexamethylphosphoramide (CASRN 680-31-9)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
	100/100, Charles River Caesarean or Charles-CD Sprague-Dawley-derived rat, 5 d/wk, 6 to 24 mo	0, 0.058, 0.293, 0.145, 0.317**	Nasal tumors rhinitis, degeneration, squamous metaplasia, and dysplasia at all doses except 0.058 mg/m^3	None	Not run	None	Lee and Trochimowicz (1982a,b,c, 1984)	

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to human equivalent dose (HED in mg/kg-day) or human equivalent concentration (HEC in mg/m^3) units. All exposure values of long-term exposure (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values for inhalation (cancer and noncancer) and oral (cancer only) are further converted to an HEC/D. Values from animal developmental studies are not adjusted to a continuous exposure.

HED = avg. mg test article/avg. kg body weight/no. daily dosed

HED_n = (avg. mg test article/avg. kg body weight/no. daily dosed)$^{1/4}$

HEC_{RESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × Regional Gas Deposition Ratio

HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood gas partition coefficient

$(HEC_{RESP})_n$ = $(EXPOSURE)_n$ × (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × Regional Gas Deposition Ratio

$(HEC_{EXRESP})_n$ = $(EXPOSURE)_n$ × (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood gas partition coefficient

[b]Not reported by the study author but determined from data.

[c]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study, NPR = Not peer reviewed.

*0.293 mg/m^3 is equivalent to 0.05 ppm for both a 1-year and 2-year exposure; 0.962 mg/m^3 is equivalent to 0.4 ppm for 10-month exposure; 8.68 mg/m^3 is equivalent to 4 ppm for 9-month exposure. See file 6 for complete HEC conversions for Lee and Trochimowicz (1982a,b,c, 1984).

**0.058 mg/m^3 is equivalent to 0.01 ppm for 2-year exposure; 0.293 mg/m^3 is equivalent to 0.05 ppm for 2-year exposure; 0.145 mg/m^3 is equivalent to 0.1 ppm for 6-month exposure, and 0.317 mg/m^3 is equivalent to 0.1 ppm for 13-month exposure.

Several studies reported in this table have uncertainties in data reporting, and critical effects confounded with pneumonia observed in all animals; thus, no NOAEL/LOAEL values could be identified in these studies.

HUMAN STUDIES

No studies investigating the effects of oral or inhalation exposure to HMPA in humans have been identified.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to HMPA have been evaluated in three subchronic-duration (i.e., Keller et al., 1997; Kimbrough and Sedlack, 1968; Shott et al., 1971), one chronic-duration (i.e., Kimbrough and Gaines, 1973), one developmental (i.e., Kimbrough and Gaines, 1966), and five reproductive studies (i.e., Shott et al., 1971; Kimbrough and Gaines, 1966; Jackson et al., 1969; Kimbrough and Gaines, 1973; Jackson and Craig, 1966).

Subchronic-duration Studies

The study by Keller et al. (1997) is selected as the principal study for deriving the subchronic and chronic p-RfDs. In a peer-reviewed study, Keller et al. (1997) evaluated the subchronic nasal toxicity of HMPA administered to rats in drinking water and by gavage. It was not stated whether the study was performed under GLP standards, but the study appears scientifically sound. The study authors first conducted a drinking water experiment in which four groups of 10 male and 10 female Charles River-CD rats obtained from Charles River Breeding Laboratories were administered HMPA (99% pure) in drinking water at doses of 0, 10, 100, 300, or 1000 ppm (equivalent to approximately 0, 1.2, 15, 42, or 123 mg/kg-day in males; 0, 2.3, 20, 63, or 229 mg/kg-day in females), 7 days/week, for 90 days. The study authors state that the animals were cared for in accordance with the *NIH Guide for Care and Use of Laboratory Animals* and observed daily for mortality and clinical signs of toxicity. Body weights, mean group food consumption, and mean group water consumption were determined weekly. After 45 and 90 days of treatment, blood samples were collected from 10 rats/sex/group for hematology (erythrocyte, leukocyte, differential leukocyte, platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration) and clinical chemistry (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities, and concentrations of blood urea nitrogen, total protein, albumin, globulin, creatinine, bilirubin, triglycerides, cholesterol, glucose, calcium, sodium, potassium, phosphate, and chloride). Urine was measured for volume, osmolality, pH, glucose, protein, bilirubin, urobilinogen, ketone, and occult blood. At the end of the treatment period, all surviving animals were sacrificed and necropsied. Selected organs were weighed (liver, spleen, kidneys, heart, testes, and brain), and histopathological examination was performed on comprehensive tissues: heart, aorta (thoracic), trachea, lungs, nose, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, femur, sternum, bone marrow (sternum), mandibular lymph nodes, mesenteric lymph nodes, spleen, thymus, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, mammary glands, ovaries, uterus/cervix, vagina, uterine horn, thyroid/parathyroid, pituitary, adrenals, brain, spinal cord, skeletal muscle, sciatic nerve, skin, eyes, exorbital lacrimal glands, and harderian glands.

No treatment-related deaths or abnormal clinical signs were observed. No effects on body weight or weight of other organs were reported in any female treatment groups or males given 10, 100, or 300 ppm. The study authors reported a significant reduction in the mean body weight of male rats administered 1000 ppm on Days 15−92 (see Appendix A, Table A.1). The authors also reported a significant reduction in absolute and relative mean testicular weights and a significant increase in relative kidney and brain weights in male rats treated with 1000-ppm

HMPA (see Appendix A, Table A.2 for relative organ weights; the data for absolute organ weights were not provided in the study). However, the increase in relative brain and kidney weights was not correlated with any histopathologic observations, and the study authors considered these finding to be spurious.

Comprehensive histopathologic examination of the controls and treated groups identified the respiratory tract and the testis as the only tissues with treatment-related lesions. The study authors reported a dose-related increase in the lesion distribution and severity in the nasal passages. In the 10-, 100-, and 300-ppm groups (both males and females), nasal lesions (epithelial denudation, regeneration, and squamous metaplasia) were limited mainly to the anterior nasal passages, whereas the general architecture of the nasal cavity was occluded by marked proliferation of the turbinate bone and myxoid fibrous tissue at 1000 ppm. The study authors rated the severity of the respiratory tract lesions as normal, minimal, mild, marked, or severe. Table A.3 presents a summary of the severity of the respiratory tract lesions in rats as presented in the study; the study did not state whether the table is for males, females, or both. The rats did not have any difficulty in breathing, despite the severe distortion of the nasal passages at 1000 ppm. Bilateral testicular atrophy occurred at 1000 ppm, and the epididymal tubules contained numerous exfoliated germ cells with scanty spermatozoa. No further details were reported on the testicular effects. The study authors identified a no-observed-adverse-effect level (NOAEL) in drinking water of 10 ppm (1.2 mg/kg-day in males and 2.3 mg/kg-day in females). Based on a significant increase in severity of nasal lesions, 100 ppm (15 mg/kg-day in males and 20 mg/kg-day in females) is considered a LOAEL. The other significant effects noted in this study were reported in males and consisted of reduced body weight and testes weights (absolute and relative), increased relative brain and kidney weights, and testicular atrophy. These effects were all noted at a higher dose (1000 ppm, or 123 mg/kg-day in males).

Based on the above drinking water study results, Keller et al. (1997) performed gavage and implant experiments. In these experiments, only male rats were used because the results of the drinking water study showed that there was no major sex-related difference in the response and the testis was a target organ. Ten Charles River-CD male rats per group were administered HMPA (99% pure) by gavage at 0, 15, 40, or 120 mg/kg-day for approximately 90 days while one group of 10 male rats received 40 mg/kg-day HMPA via osmotic minipumps that were implanted subcutaneously in the backs of the rats. The minipump was designed to deliver approximately 2.5 µL/hour over 28 days, and the minipump was removed after each 28-day period and replaced with a new pump over the course of the study (approximately 90 days). The study authors designed the gavage doses to mimic the dosages received by males in the drinking water study, whereas in the implant study, the pump was designed to deliver a dosage of 40 mg/kg-day over the course of the study without the possibility of direct contact of HMPA with the nasal epithelium. The animals were observed daily for clinical signs of toxicity, and body weights were taken weekly. Five rats per group were sacrificed after 45 days, and the remaining rats in each group were sacrificed after 85 days. Clinical laboratory evaluations of the blood and urine were not performed in this experiment; the brain, liver, and testes were weighed at necropsy. The nasal passages, liver, epididymides, prostate, seminal vesicles, and testes were collected for light microscopic examination and were examined in the 0- and 120-mg/kg-day dose groups, whereas only the nasal passages and testes were examined in the 15- and 40-mg/kg-day groups.

Gavage treatment with HMPA in males at 120 mg/kg-day resulted in a statistically significant ($p < 0.05$) decrease in mean body-weight gain (Keller et al., 1997). In addition, testicular atrophy was observed at this dose, with the same qualitative severity as the atrophy noted in the 123-mg/kg-day dose group in the drinking water study. No body-weight changes were observed at 15 or 40 mg/kg-day. Similar to the drinking water study, nasal lesions were observed in males at all dose groups. These were qualitatively and quantitatively similar to those seen in the drinking water study (see Appendix A, Table A.4). Similarly, nasal lesions were found in rats in the implant study at 40 mg/kg-day—except there were no significant adhesions in the ethmoturbinates—so the study authors considered these lesions to be less severe than those seen in the drinking water and gavage studies. The study authors did not identify a NOAEL or LOAEL from the gavage or implant studies. However, 15 mg/kg-day is considered a LOAEL based on nasal lesions noted in the gavage study, and there is no NOAEL because these lesions were observed at the lowest dose.

Kimbrough and Sedlak (1968) investigated the morphology of lung lesions in rats exposed to HMPA orally. They conducted a study in which a group of 20 male Sherman strain rats (11 weeks old) was given HMPA (technical grade mixed into ground Purina Chow) at 2000 ppm for 52−72 days, corresponding to an average dietary intake of 106−127 mg/kg-day; one group was fed Purina Chow and kept as a control. The average dietary intake was calculated by the study authors based on the average food intake, which was stated to be 52.8−63.4 g/kg. No additional information was provided on doses by the study authors, so it is not known how many doses were administered. Food consumption of the animals was determined during the first, second, sixth, and seventh week of the study. The animals were observed daily for mortality and clinical signs of toxicity. After treatment, the lungs were removed, weighed, and necropsied. The study authors performed a microscopic examination on one or two sections from each lobe of the lungs and a section of the trachea. Four of the treated rats died during the study (one rat each died on Day 24, Day 52, Day 68, and Day 70 of exposure). Eight additional rats from the treated group and 10 from the control group were sacrificed after 52 days, while the remaining surviving animals (10) were sacrificed on Day 72 of exposure. Signs of toxicity observed throughout the treatment included weight loss, rapid and irregular breathing, and wheezing. Some rats had nasal discharge. The upper respiratory system and trachea of both the control and treated group were normal—except for some mucus in the trachea of a few (number unspecified) rats in the treated group. A statistically significant increase ($p \leq 0.001$) in lung weights when compared with controls was stated by the authors to be "1.235% against 0.435% of body weight"; however, the meaning of this statement is not clear. No further details were provided by the study authors on the increase in lung weight, and it is unclear whether this finding represents an absolute or relative increase in lung weight. The pathological examination of the lungs revealed lung lesions, such as bronchitis, bronchiectasis, consolidation, and abscess formation, which were extended from one lobe to several lobes. In addition, pneumonic changes or presence of macrophage cells were observed. Other pathological findings included periarteritis, fibrosis, and asquamous metaplasia. Based on the study design (only one dose was applied), no NOAEL/LOAEL could be determined.

In a 92-day study conducted by Shott et al. (1971), 20 albino rats per sex per group were administered HMPA by gastric intubation for 7 days/week at doses of 0, 2, 10, or 50 mg/kg-day. Body weights, appearance, and behavior were recorded daily, and food consumption data were recorded weekly. The study authors evaluated hematology (erythrocyte count, total and differential leukocyte counts, and hematocrit and hemoglobin determinations), biochemistry

(blood urea nitrogen, serum glutamic-pyruvic transaminase, and fasting blood sugar), and urinalysis (color, pH, specific gravity, sugar, albumin, acetone, protein, bilirubin, occult blood, and microscopic examination of the sediment) for randomly selected animals (5/sex/group). They also performed necropsies at the end of the treatment period. A complete histopathology evaluation of the brain, pituitary, thyroid, lung, liver, spleen, heart, kidney, adrenal, stomach, pancreas, small and large intestine, ovary or testis, urinary bladder, bone, and bone marrow was performed on animals from the control and 50-mg/kg-day groups, whereas, in the 2- and 10-mg/kg-day groups, samples of thyroid, lung, liver, kidney, testis or ovary, and bone marrow were examined.

The number of rats surviving in each of the three dose groups (2, 10, and 50 mg/kg) was reportedly similar (exact numbers were not reported). No effects were noted in the 2-mg/kg-day group; biochemical, hematologic, and urinalysis parameters were comparable between the control and test groups. The only clinical effect noted in the 10-mg/kg-day group was a statistically significant decrease (percentage decrease not provided) in thyroid weight in females and "growth suppression" (further details as to whether in males or females and percentage not provided). Treatment-related effects were primarily observed at 50 mg/kg-day as evidenced by a greater incidence (incidence not provided) of respiratory disease, a statistically significant decrease in growth rate, and a reduction in food consumption (further details not provided). Pathological examination revealed lesions of respiratory disease (severe manifestations of chronic murine pneumonia) in the 50-mg/kg-day group, whereas, in the control, 2-, and 10-mg/kg groups, only mild pneumonic changes were noted. In addition, at 50 mg/kg-day, a slight increase in splenic extramedullary hematopoiesis was noted (sex not reported), and eosinophilic, fine-droplet material was noted in the cytoplasm of renal epithelial cells of male rats only at 50 mg/kg-day. No other compound-related morphologic or pathologic changes were observed in the other tissues. The study authors noted a statistically significant dose-related decrease (percentage not provided) in thyroid weights in females at 10 and 50 mg/kg-day, liver weights in females at 50 mg/kg-day, and kidney weights in both males and females at 50 mg/kg-day. Although the organ weights were decreased, the organ-to-body weight ratios were comparable; thus, the study authors suggested that these changes probably reflect the decreased total body weights rather than a detrimental organ-specific activity of the compound. Although the study authors did not identify a NOAEL or LOAEL from this study, 10 mg/kg-day is considered a LOAEL based on a statistically significant decrease in thyroid weight in females, and 2 mg/kg-day is considered a NOAEL.

Chronic-duration Studies

Kimbrough and Gaines (1973) investigated the chronic effects of low HMPA dietary intake in male Sherman rats. Four groups of 15 male rats were fed HMPA in their diet at doses of 0, 0.78, 1.56, 3.12, or 6.25 mg/kg-day for 2 years. The study was performed in two parts, each consisting of two treated groups and one control group. Data reporting was poor, and no statistical analyses were done on this study. Pathological examination was performed on all treated animals at the end of the study. After 1 year of dosing, evidence of periarteritis in different organs was observed in all treated and control rats; however, the authors did not consider this finding significant because it was seen in both treated and control rats. The incidence of lung disease, primarily related to an inflammatory process, was generally increased (no statistics were done, see Appendix A, Table A.5 for numbers of animals) in the exposed animals compared to the controls. This finding was confirmed by microscopic examination, which showed evidence of bronchitis, peri-bronchitis, bronchiectases, bronchopneumonia,

abscess formation, or fibrosis. Tumor incidences in either reticulum cell or lymphosarcoma of the lungs (not included in the tabulation of the lung disease) were observed occasionally. This tumor incidence, however, was low in the treated animals and appears to be comparable to the control incidence (see Appendix A, Table A.5). Kidney disease was also observed in the different groups but did not appear to be dose related. The study authors did not indicate a NOAEL or LOAEL in this study; due to the poor data reporting and lack of statistical analysis, no NOAEL or LOAEL is identified.

Developmental Studies

Kimbrough and Gaines (1966) administered 200 mg/kg-day HMPA by gavage to 10 female Sherman rats on Day 7 prior to mating and continuing until GD 20. Eight female rats were dosed daily with tap water and served as controls. The females were sacrificed, and their uteri were examined. The study authors also examined the offspring and the placentae, and the weight of each offspring was recorded. Abnormalities were not observed in the offspring of either the treated or the control females. The weight of each fetus and placenta, the number of animals per litter, and the number of resorption sites in the treated animals were comparable to the controls. No further details are provided on this study. The study authors did not indicate a NOAEL or LOAEL, and neither is identified because only one dose was tested in this study and the data reporting was poor.

Reproductive Studies

Shott et al. (1971) performed a two-generation reproductive toxicity study in rats with HMPA (purity >99%), which was administered at 0, 2, or 10 mg/kg-day, to groups of 80 young adult albino male and female rats. Animals were randomized into three equal groups and designated as the parents (P1) of the first generation offspring ($F1_A$, $F1_B$, and $F1_C$ litters). The tested animals received HMPA daily via gavage for 100 days prior to breeding and through the breeding and weaning period of the $F1_A$ litters (total study period of 169 days). P1 females were rebred to produce the $F1_B$ and the $F1_C$ litters, but no HMPA was administered to any animals in this period. After a 21-day nursing period, 20 male and 20 female rats were selected from the F_{IA} control and test groups and designated as the P2 animals. The P2 animals were mated, resulting in a F2 generation. Observations made during the study include mortality, clinical signs of toxicity, body weight (weights of the pups were recorded by sex for each filial group at 24 hours and at weaning), mating and fertility indices (FI), gestation indices (GI), lactation indices (LI), and live birth indices (LBI). Necropsy was performed on one-half of each litter from the $F1_B$ generation, which were sacrificed immediately after weaning. Symptoms of pneumonia were observed among the P1 test animals. However, the incidence of pneumonia in the test rats decreased and became comparable to the incidence in controls after weaning of the $F1_A$ litters and compound withdrawal.

Treatment with HMPA had no effect on GI, LBI, or LI reproduction indices (see Appendix A, Table A.6). However, the FI corresponding to $F1_B$ litters was reduced at both doses. The study authors indicated that this change appeared to be a consequence of the pneumonia that was observed in the P1 test animals, rather than of HMPA administration. This is supported by the fact that this effect was not observed in FI for the $F1_A$ and $F1_C$ litters (see Appendix A, Table A.6) and the reproduction of the $F1_A$ animals was normal, as indicated by the reproduction indices of the P2 animals, which were derived from the F_{IA} litter. No teratogenic effects were observed at any dose. The study authors noted a dose-related slight depression of weight gain and food consumption in P1 animals (during the premating period) and P2 females.

This finding was not considered a meaningful effect by the study authors because the survival of the treated animals during the entire experiment was comparable to that of the controls. The study authors concluded that reproduction or teratogenicity in rats was not affected at these dosages. Although, the study authors did not indicate a NOAEL or LOAEL for this study, 10 mg/kg-day is considered a NOAEL based on the lack of reproductive and teratogenic effects observed at this dose. The reduction in the fertility index is not considered a reproductive effect resulting from HMPA administration because the authors stated that this reduction was the result of the pneumonia that occurred in the P1 rats.

Kimbrough and Gaines (1966) conducted a number of reproductive toxicity studies on HMPA in rats. Very limited information is provided, and the data reporting is poor. In several experiments, the study authors investigated the effect of single and multiple doses of HMPA on organs of the male rat reproductive system. Another experiment evaluated whether HMPA exposure affected female rat reproductive organs. The age of the animals was not provided for any of these studies.

In a preliminary experiment, HMPA was administered by gavage to male Sherman rats (number not provided) at a dose of 25 mg/kg-day for 56 days. The study authors stated that HMPA did not affect the fertility of male rats and the testes appeared to be normal in size. No further details were provided.

In the next experiment, one group of adult male Sherman rats (the number of animals varied per dose, see Appendix A, Table A.7) received a single dose of 0, 500, 1000, or 2000 mg/kg-day of HMPA by gavage and was sacrificed 40 days after receiving the dose. A second group received repeated gavage doses of 0, 100, 200, or 400 mg/kg-day HMPA for 36−99 days (the number of animals and the number of doses varied and are listed in Appendix A, Table A.8). Pathological examination of the testes was performed on both groups of rats. In the first group, partial or complete testicular atrophy was not observed in the control or in the 500-mg/kg-day rats—but it was noted in two rats given 1000 mg/kg-day and nine rats given 2000 mg/kg-day. In the second group, partial or complete testicular atrophy was not observed in the control rats, but it was observed in 6/10 rats dosed at 100 mg/kg-day, 4/5 rats dosed at 200 mg/kg-day, and 5/8 rats dosed at 400 mg/kg-day. A number of rats also developed pneumonia (see Appendix A, Table A.8).

In another experiment, male Sherman rats (number of animals varied) were given HMPA for 61−103 days in the diet at 750 ppm, corresponding to doses ranging from 40−80 mg/kg-day. Control rats were given Purina chow only for 45−103 days. No further information was provided by the study authors on data obtained during the study. Testicular atrophy and pneumonia were observed in all treated animals (no details on the pathological examination were reported). The study authors concluded that HMPA had the same effect on the testes whether it was given in the diet or by gavage.

Kimbrough and Gaines (1966) also examined whether HMPA would affect the organs of the female rat reproductive system. A group of six female Sherman rats was given a single dose of 2500 mg/kg-day HMPA (route not provided). The animals were sacrificed 36 days after dosing. No further information was provided on this study. The study authors only reported that no effects on the female reproductive organs were observed in this experiment.

In summary (from all the Kimbrough and Gaines, 1966 experiments), the study authors stated that HMPA has a very specific effect on the testes in rats when fed at a dietary level of 750 ppm. The study authors did not specifically indicate a NOAEL or a LOAEL, and values are not identified from these studies due to poor data reporting and lack of details about the studies.

Jackson et al. (1969) investigated the effect of HMPA on male fertility in Wistar rats. Doses of 25, 50, or 100 mg/kg-day HMPA were administered in drinking water to male rats for 3 weeks (number of animals varied). In addition, eight male rats were administered HMPA for 6 days only at a dose of 500 mg/kg-day (HMPA further purified by gas-liquid chromatography; purity of original HMPA or further purified HMPA not provided). Histological examination was carried out on the reproductive organs (testes and epididymides), and fertility was determined based on examining the number of litters delivered. Body-weight changes and white blood cell count were also recorded.

No changes in body weights or in white blood cell counts were observed. No effect on fertility (based on average litter size) was noted at 25 mg/kg-day; however, at 50, 100, and 500 mg/kg-day, there was a decrease in litter size. A 50% decrease in testis weight for rats given 100 mg/kg-day HMPA was observed, whereas administration of 500 mg/kg-day for 5 to 6 days was associated with changes in the testes of all animals. No significant damage to the testes was noted at 50 mg/kg-day, whereas severe damage to the testes was noted by the end of treatment (3 weeks) with 100 mg/kg-day and by 5 to 6 days of treatment with 500 mg/kg-day. Most of the tubules were depopulated, and giant cells were frequent. No histological changes were observed in the pituitaries of rats examined at the end of treatment. Multiple lung abscesses (no further information provided) in the rats administered 100-mg/kg-day HMPA for 6 weeks were noted. The study authors concluded that the mechanism of action of HMPA on spermatogenic cells consisted of producing aspermia following destructive effects on spermatids and spermatocytes, which could result in protracted sterility. The study authors did not identify a NOAEL or LOAEL from this study. Due to the lack of a control group and the lack of study details reported, a NOAEL or LOAEL is not identified.

Kimbrough and Gaines (1973) conducted an additional study on reproduction in which 15 male Sherman rats were administered a single dose of 6.25 mg/kg HMPA in the diet and were mated 6 and 12 months after the single dose. Fifteen rats served as controls. No effect on reproduction was observed at this dose of HMPA. Microscopic examinations revealed testicular atrophy that, according to the study authors, probably occurred late in life and was the result of age and debilitating disease (pneumonia). The study authors did not identify a NOAEL or LOAEL from this study. Due to the lack of study details reported, a NOAEL or LOAEL is not identified.

Jackson and Craig (1966) administered male rats (strain and number not reported) 21 daily doses of 100 mg/kg-day, 5 daily doses of 250 mg/kg-day, or 6 daily doses of 500 mg/kg-day HMPA by mouth (no further details provided). Mice (strain and number not reported) were administered 6 daily doses of 500 mg/kg-day, and rabbits (strain and number not reported) were administered 10 daily doses of 100 mg/kg-day. It was not stated when the doses were administered, although the study authors stated that there was a 12-week mating period for the rats. The average weekly litter size from male rats and mice and the sperm count in rabbits after 2 weeks of HMPA administration were assessed. The study authors reported that the minimum effective dose for "reversible episodes of sterility" was 100 mg/kg-day for the rat,

apparently based on the litter size. After about 5 weeks, the rabbits became nearly aspermic for 3 weeks, while the volume of seminal fluid remained with normal limits. The study authors identified 100 mg/kg-day as the LOAEL for "reversible episodes of sterility"; however, based on the lack of a control group and lack of study details, including the numbers of animals, a NOAEL or LOAEL is not identified.

Inhalation Exposures

The effects of inhalation exposure of animals to HMPA have been evaluated in one carcinogenicity study (Lee and Trochimowicz, 1982a,b,c, 1984). These are redundant publications by the same authors. They contain nearly identical text relative to the experimental design with slightly different interpretations of the data.

Chronic-duration Studies

Lee and Trochimowicz (1982a,b,c, 1984) studied the effects of inhalation exposure of rats to HMPA for 3 to 24 months. In two separate experiments, the study authors exposed Charles River Caesarian-derived (ChR-CD) rats (Lee and Trochimowicz, 1982c, 1984) or Charles-CD Sprague-Dawley-derived (Lee and Trochimowicz, 1982a,b) to HMPA vapor at doses of 0, 10, 50, 100, 400, or 4000 ppb (v/v). Table A.9 provides an overview of exposure protocol. The study authors were not consistent when referring to rat strains from the same experiment. It was not stated whether GLP was followed in these studies.

In the first part, four groups of 120 rats per sex per group were exposed to 0, 50, 400, or 4000 ppb (v/v) (equivalent to 0, 0.37, 2.9, or 29 mg/m^3 (as calculated by IARC [1999] and verified based on conversion of ppb to mg/m^3) HMPA (purity 99.9%) for 6 hours/day, 5 days/week, for 3 to 24 months. One control group for each sex was used. Rats exposed to 50 ppb were further divided into groups of 60 rats/sex/per group. One group of each sex was exposed to 50 ppb for 12 months and placed in holding rooms. The other two groups were exposed continuously for up to 24 months; some animals were sacrificed at 3, 8, and 12 months, and the remaining animals were sacrificed at 24 months. One group of 120 males and 120 females was exposed to 400 ppb for 10 months, and one group of 120 males and 120 females was exposed to 4000 ppb for 9 months; both dose groups were held and sacrificed at 24 months, except for 18 rats from each dose group that were sacrificed after 3 months of exposure. Control rats were sacrificed after 24 months with interim sacrifices performed at 3 and 12 months (see Appendix A, Table A.9).

In the second part, the investigators exposed 100 rats per sex per group to 0, 10, 50, or 100 ppb (v/v) (equivalent to 0, 0.073, 0.37, or 0.73 mg/m^3 [HMPA as calculated by IARC, 1999 and verified based on conversion of ppb to mg/m^3]). All of the rats exposed to 10 ppb were exposed for 24 months and then sacrificed. Twenty of the rats exposed to 50 ppb were exposed for 12 months and then sacrificed, and the remaining were exposed for 24 months and then sacrificed. Fifty of the 100 males and 50 of the 100 females exposed to 100 ppb were exposed for 6 months, and the remaining males and females in this group were exposed for 13 months; all rats were held until 24 months and then sacrificed. Control rats were sacrificed after 24 months. Experimental design was inadequately reported, and the summary in Table A.9 is believed to be accurate with respect to dosing and time of sacrifice, although the number of animals sacrificed at a particular time is not clear.

Microscopic examination was carried out on tissues from all the rats (from the two experiments)—except for those exposed to 50 ppb in the second experiment. The lung, trachea, thyroid, pituitary, adrenal, testis, and nose were examined by light and electron microscopy. In addition, three coronal sections from the anterior nasal cavity and two coronal sections from the posterior nasal cavity were examined by light microscopy. No clinical analysis was performed, and the study authors did not record body weights during the study.

Results from pathological and microscopic examination showed dose-related nasal tumors, rhinitis, degeneration, squamous metaplasia, and dysplasia in the nasal epithelium at 50, 100, 400, and 4000 ppb, but no HMPA-related lesions were found at 10 ppb. According to the study authors, nasal tumors were first detected after 7 months exposure at 400 and 4000 ppb, after 9 months at 100 ppb, and after 12 months at 50 ppb (no interim sacrifices were performed at 7 and 9 months, and the study authors did not state how the nasal tumors were detected in rats at these exposure periods). The tumor incidence was 20% at 50 ppb after 24 months exposure, 56% at 100 ppb after 13 months exposure, 82% at 400 ppb after 10 months exposure, and 83% at 4000 ppb after 9 months of exposure (see Appendix A, Table A.10). However, the tumor data presented in Table A.10, which are adopted from the authors' original publications (Lee and Trochimowicz, 1982a,b), are not easily aligned with the length of exposure and time of sacrifice reported in the experimental design. For example, Table A.10 shows a tumor rate of 37.5%, or 75/200 animals, for the 100-ppb group, which places into one group both the 100 animals exposed for 6 months and the 100 animals exposed for 13 months. It is also unclear which 194 animals are included in Table A.10 in the 50-ppb group. It might be assumed that these are from the 200 animals dosed at 50 ppb in the second experiment; however, some of these animals had an interim sacrifice at 12 months. In summary, it is difficult to align the summary tumor data reported by the authors (see Appendix A, Table A.10) with the numbers of animals used in each experiment and the sacrifice times (see Appendix A, Table A.9). There are no additional tables provided in any of the authors' original publications (Lee and Trochimowicz, 1982a,b,c, 1984) that clarify this issue. Most nasal tumors occurred in the respiratory epithelium of the anterior nasal cavity and invaded ventral nasal bone or the posterior nasal cavity, and most of the nasal tumors were squamous cell carcinomas. The incidence and severity of the nasal lesions were dose related, but marked differences in the severity of the lesions were observed among individual rats in the same group. The nasal tumors (total of 473) were characterized as epidermoid carcinoma (71.9%), adenoid squamous carcinoma (15%), papilloma (8.2%), transitional (respiratory epithelial) carcinoma (1.9%), adenocarcinoma (1.3%), undifferentiated tumor (1.1%), (mixed) pleomorphic tumor (0.4%), and adenomatous polyp (0.2%) (see Appendix A, Table A.10). Results were not differentiated by sex.

A dose-related increase in the incidence and severity of tracheitis, degeneration of the tracheobronchial epithelium, and murine pneumonia was noted at 100, 400, and 4000 ppb. No significant differences were noted, however, in the lesions at 10 and 50 ppb compared to the control group. The study authors concluded that because no primary lesions attributable to HMPA were observed in the alveoli, the pneumonia appeared to be a secondary lesion caused by infectious agents after the destruction of the mucociliatory apparatus by HMPA in the air passages.

The study authors noted that the toxic effects of HMPA by inhalation were mostly observed in the upper respiratory air passages with markedly decreased toxic effects in the lower air passages, without any effects on the alveolar walls. They further indicated that this finding is

expected because HMPA is water soluble and its inhaled vapor appears to be absorbed mostly by the respiratory epithelium of the anterior nasal cavity, failing to provoke tissue response in the lower respiratory passages and alveoli (Lee and Trochimowicz, 1982a,b,c, 1984).

Subchronic-duration Studies

No studies could be located regarding the effects of subchronic inhalation exposure of animals to HMPA.

Developmental and Reproduction Studies

No studies could be located regarding the effects of inhaled HMPA on reproduction and fetal development.

Other Exposures

The effects of dermal exposure of animals to HMPA have been evaluated in one subchronic-duration study (i.e., Shott et al., 1971).

Subchronic-duration Studies

Shott et al. (1971) applied HMPA to the skin of rabbits (10/sex/group) at doses of 100 or 500 mg/kg-day for 6 hours per day, 5 days per week, for 3 weeks. Observations made during the study include clinical signs of toxicity, dermal irritation, body-weight changes, and necropsy observations. The study authors also assessed clinical chemistry. Dose-related alterations attributable to the compound were weight loss, altered gastrointestinal function, including sporadic anorexia and transient diarrhea, intermittent anuria, and incoordination. Dermal alterations including erythema and desquamation were noted at both 100 and 500 mg/kg-day, and dermal atonia (in addition to erythema and desquamation) was also noted at 500 mg/kg-day. No differences were noted in the clinical chemistry results between the treated and control animals. Other than the dermal changes, necropsy examination did not reveal consistent alterations attributable to the dermal administration of HMPA.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

A few studies on the short-term toxicity, toxicokinetics, and genotoxicity of HMPA are available.

Short-term Studies

In two separate experiments, Kimbrough and Gaines (1966) evaluated the acute oral toxicity of HMPA in rats. In the first experiment, male and female Sherman strain rats were given a single dose of technical grade HMPA (dissolved in water) by stomach tube or mixed in their diet. The number of animals was not reported, and only the lowest dose tested was listed in the study (500 mg/kg-day for males and 2000 mg/kg-day for females). Complete autopsies were done on most animals, and a blood sample was taken for white blood cell count. The main clinical signs observed in the rats included involuntary urination, mild muscle fasciculation, convulsions, and bloody urine. HMPA was found to be more toxic to male rats than females. The acute oral LD_{50} of HMPA for male rats was identified as 2650 mg/kg-day; for female rats, the value was 3360 mg/kg-day. To further investigate the acute tissue changes, the study authors carried out a second experiment in which six adult male rats were given a single dose of 2500 mg/kg-day HMPA and sacrificed 3 days after dosing. The study authors performed a pathological examination of the rat tissues. They observed two instances of necrotizing cystitis and four instances of blood cells and hyaline casts in the rat kidney tubules. Intra-alveolar

hemorrhage and bronchiectasis were observed in three rats. All of the treated rats had smaller spleens than normal, and two of the rats had a reduced number of sperms and spermatids and occasionally multinucleated testicular cells.

Toxicokinetics

Metabolism studies show that HMPA is metabolized via successive *N*-demethylation, yielding pentamethylphosphoramide (PMPA), *N*,*N*,*N*',*N*''-tetramethyl-phosphoramide, and *N*,*N*',*N*''-trimethylphosphoramide (TriMPA). At each demethylation stage, the cytochrome p-450-mediated reaction yields unstable methylol intermediates that decompose to the demethylated phosphoramides with the release of formaldehyde, see Figure 2 (Jones and Jackson, 1968; Dahl and Brezinski, 1985).

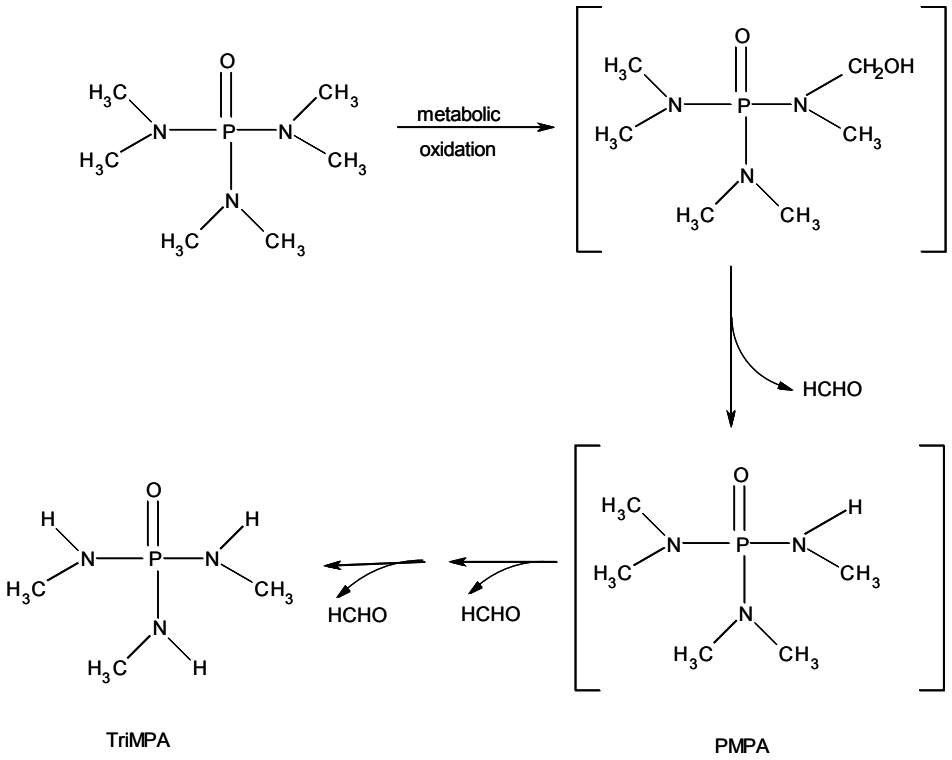

Figure 2. Hexamethylphosphoramide Metabolism

Genotoxicity

The genotoxicity (e.g., clastogenicity, mutagenicity) of HMPA has been tested in a number of in vitro (see Table 3) and in vivo studies (see Table 4), with mixed results. Most of the study results presented in Tables 3 and 4 were obtained through existing summaries, and few original sources were available for review.

As reported by IARC (1999) (see Table 3 for the primary references cited in IARC), HMPA gave primarily negative results in the Ames mutagenicity test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the presence and absence of metabolic activation systems. It was positive in one study in several strains of *Salmonella*

typhimurium with metabolic activation in a liquid suspension test. It also was negative in several studies on *Escherichia coli* WP2 and WP2μvrA, with one study giving positive results with metabolic activation. It was positive and weakly positive in the yeast *Saccharomyces cerevisiae* and gave positive results in *Drosophila melanogaster* for somatic mutation and mitotic recombination.

As reported by IARC (1999) (see Table 3 for the primary references cited in IARC), HMPA gave mixed results in mammalian cells, with positive results for DNA-protein cross links in rat nasal epithelial cells and for gene mutations in mouse lymphoma P388F and L5178Y cells, with metabolic activation. In two studies, hexamethylphosphoramide was negative for sister chromatid exchange in Chinese hamster ovary cells, both with and without metabolic activation, while it was positive, with metabolic activation, in an additional two studies for the same endpoint. In human hepatoma HepG2 cells, it was positive for the micronucleus test, while it was negative in human lymphocytes for the micronucleus test and for chromosomal aberrations.

In vivo studies using intraperitoneal (i.p.) exposure showed mixed results. As reported by IARC (1999) (see Table 4 for the primary references cited in IARC), HMPA induced sister chromatid exchange in CBA/J mouse bone marrow but not in mouse liver. It was positive in the micronucleus test in mouse and rat bone marrow, but it was negative for chromosomal aberrations in mouse bone marrow. One study was positive and one was negative for the dominant lethal test in mice, and it was negative or inconclusive for sperm morphology in mice. HMPA, when administered by gavage to male Han Wistar rats, showed statistically significant increases in chromosome aberrations in peripheral lymphocytes after 15 and 28 days of dosing (Hayes et al., 2009) and induced micronuclei in the bone marrow after 14 and 28 days of dosing (Doherty et al., 2009).

Table 3. Genotoxicity Studies of Hexamethylphosphoramide In Vitro

Test System	Endpoint	Results[a] without Activation	Results[a] with Activation	Dose[b] (µg/mL)	Reference
Salmonella typhimurium	Forward mutation, 8-azaguanine	NT	-	1000	Skopek et al. (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	NG	Baker and Bonim (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	1000	Brooks and Dean (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538.	Reverse mutation	-	-	NG	Garner et al. (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100	Reverse mutation (fluctuation test)	-	-	500	Hubbard et al. (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1537	Reverse mutation	-	-	2500 (TA1537); 5000 (TA98, TA100)	MacDonald (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1537, TA1538	Reverse mutation	-	-	NG	Martire et al. (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1537, TA1538	Reverse mutation	-	-	NG	Nagao and Takahashi (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	500	Richold and Jones (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	1000	Rowland and Severn (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	NG	Simmon and Shepherd (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA98, TA100	Reverse mutation	-	-	NG	Venitt and Crofton-Sleigh (1981) (as cited in IARC, 1999)
Salmonella typhimurium TA97, TA98, TA100, TA1535	Reverse mutation	-	-	5000	Zeiger and Haworth (1985) (as cited in IARC, 1999)

Table 3. Genotoxicity Studies of Hexamethylphosphoramide In Vitro

Test System	Endpoint	Results[a] without Activation	Results[a] with Activation	Dose[b] (µg/mL)	Reference
Salmonella typhimurium TA97, TA98, TA100, TA1537	Reverse mutation, liquid suspension test	-	+	2000 (TA100); 5000 (TA97); 10,000 (TA98, TA1537)	Sarrif et al. (1997) (as cited in IARC, 1999)
Salmonella typhimurium TA1535	Reverse mutation, liquid suspension test	-	-	40000	Sarrif et al. (1997) (as cited in IARC, 1999)
Escherichia coli K-12/343/113	Forward or reverse mutation	-	-	4000	Mohn et al. (1981) (as cited in IARC, 1999)
Escherichia coli WP2 and WP2µvrA	Reverse mutation	-	+	NG	Venitt and Crofton-Sleigh (1981) (as cited in IARC, 1999)
Escherichia coli WP2µvrA (fluctuation test)	Reverse mutation	-	-	1000	Gatehouse (1981) (as cited in IARC, 1999)
Escherichia coli WP2, WP2µvrA, WP2µvrApKM101	Reverse mutation	-	-	NG	Matsushima et al. (1981) (as cited in IARC, 1999)
Saccharomyces cerevisiae JD1	Homozygous by mitotic gene conversion	-	+	50	Sharp and Perry (1981) (as cited in IARC, 1999)
Saccharomyces cerevisiae D7	Homozygous by mitotic gene conversion	-	+	2000	Zimmermann and Scheel (1981) (as cited in IARC, 1999)
Saccharomyces cerevisiae DEL	Homozygous by mitotic gene conversion	(+)	(+)	50,000	Carls and Schiestl (1994) (as cited in IARC, 1999)
Saccharomyces cerevisiae XV-185-14C	Reverse mutation	-	(+)	100	Mehta and Von Borstel (1981) (as cited in IARC, 1999)
Schizosaccharo-myces pombe	Forward mutation, five loci	-	-	30	Loprieno (1981) (as cited in IARC, 1999)
Drosophila melanogaster, white/white eye mosaic test	Somatic mutation and mitotic recombination	+		18 feed	Vogel and Nivard (1993) (as cited in IARC, 1999)
Drosophila melanogaster, white/white eye mosaic test	Somatic mutation and mitotic recombination	+		18 feed	Aguirrezabalaga et al. (1994) (as cited in IARC, 1999)

Table 3. Genotoxicity Studies of Hexamethylphosphoramide In Vitro

Test System	Endpoint	Results[a] without Activation	Results[a] with Activation	Dose[b] (µg/mL)	Reference
Drosophila melanogaster, white/ivory eye test	Somatic mutation and mitotic recombination	+		9-ppm feed	Ferreiro et al. (1995) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations	+		250-ppm feed	Valencia and Houtchens (1981) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations	+		100-ppm feed	Vogel et al. (1981) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations	+		100-ppm feed	Wurgler and Graf (1981) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations, heritable translocation test, chromosome loss (ring-X)	+		25-ppm feed (lethal mutations, translocation), 11-ppm feed (chromosome loss)	Vogel et al. (1985) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations and heritable translocation test	+		100-ppm feed	Foureman et al. (1994) (as cited in IARC, 1999)
Drosophila melanogaster	Sex-linked recessive lethal mutations	+		45-ppm feed	Aguirrezabalaga et al. (1995) (as cited in IARC, 1999)
Drosophila melanogaster	Survival of DNA repair-deficient mus homozygotes relative to their repair-proficient heterozygous siblings	+		896-ppm feed	Henderson and Grigliatti (1992) (as cited in IARC, 1999)
Rat nasal epithelial cells	DNA-protein cross-links	+	NT	179	Kuykendall et al. (1995) (as cited in IARC, 1999)
Chinese hamster ovary cells	Gene mutation, five loci	-	-	31,000	Carver et al. (1981) (as cited in IARC, 1999)
Chinese hamster lung V79 cells	Gene mutation, hprt locus	-	In.	200	Knaap et al. (1981) (as cited in IARC, 1999)

Table 3. Genotoxicity Studies of Hexamethylphosphoramide In Vitro

Test System	Endpoint	Results[a] without Activation	Results[a] with Activation	Dose[b] (µg/mL)	Reference
Mouse lymphoma P388F cells	Gene mutation, tk locus	NT	+	8.28	Anderson and Cross (1985) (as cited in IARC, 1999)
Mouse lymphoma L5178Y cells	Gene mutation, tk locus	-	+	1500	Jotz and Mitchell (1981) (as cited in IARC, 1999)
Chinese hamster ovary cells	Sister chromatid exchange	-	-	1000	Evans and Mitchell (1981) (as cited in IARC, 1999)
Chinese hamster ovary cells	Sister chromatid exchange, chromosomal aberrations	-	-	339	Natarajan and Van Kesteren-Van Leeuwen (1981) (as cited in IARC, 1999)
Chinese hamster ovary cells	Sister chromatid exchange	-	+	10	Perry and Thomson (1981) (as cited in IARC, 1999)
Chinese hamster ovary cells	Sister chromatid exchange, Micronucleus test	-	+[c]	5.4 (sister chromatid exchange), 2.7 (micronucleus test)	Darroudi and Nataranan (1993) (as cited in IARC, 1999)
Rat liver RL_1 cells	Chromosomal aberrations	-	NT	100	Dean (1981) (as cited in IARC, 1999)
Human hepatoma Hep G2 cells	Micronucleus test	+	NT	1.6	Natarajan and Darroudi (1991) (as cited in IARC, 1999)
Human hepatoma Hep G2 cells	Micronucleus test	+[d]	NT	0.5	Darroudi et al. (1996) (as cited in IARC, 1999)
Human lymphocytes	Micronucleus test	-	NT	1.8	Darroudi et al. (1996) (as cited in IARC, 1999)
Human lymphocytes	Chromosomal aberrations	-	NT	900	Chang and Klassen (1968) (as cited in IARC, 1999)

[a]+, positive; (+), weak positive; - negative; NT, not tested; In, inconclusive.
[b]LED, lowest effective dose; HID, highest ineffective dose; NG, not given.
[c]Activation system using human hepatoma (Hep62) S-9; rat liver S-9 was negative.
[d]The fluorescent in situ hybridization assay shows that approximately 80% of the micronuclei are centromere-positive compared to approximately 50% in controls.

Table 4. Genotoxicity Studies of Hexamethylphosphoramide In Vivo

Test System	Endpoint	Results[a]	Dose (mg/kg-day)[b]	Reference
CBA/J mouse bone marrow	Sister chromatid exchange	+	15.4 i.p. × 1	Paika et al. (1981) (as cited in IARC, 1999)
CBA/J mouse liver	Sister chromatid exchange	-	1304 i.p. × 1	Paika et al. (1981) (as cited in IARC, 1999)
Han rat bone marrow	Micronucleus test	+	100 gavage (28 days)	Doherty et al. (2009)
B6C3F1 mouse bone marrow	Micronucleus test	+	1232 i.p. × 2	Salamone et al. (1981) (as cited in IARC, 1999)
ICR mouse bone marrow	Micronucleus test	+	205 i.p. × 2	Kirkhart (1981) (as cited in IARC, 1999)
CDI mouse bone marrow	Micronucleus test	+	205 i.p. × 2	Tsuchimoto and Matter (1981) (as cited in IARC, 1999)
C57BL/6J mouse bone marrow	Micronucleus test	+	1850 i.p. × 2	Richardson et al. (1983) (as cited in IARC, 1999)
C57BL/6J, C3H/C57, BALB/c/CBA mouse bone marrow	Micronucleus test	+	1315 i.p. × 2	Styles et al. (1983) (as cited in IARC, 1999)
Alderley Park rat bone marrow	Micronucleus test, chromosomal aberrations	+	1850 i.p. × 1	Albanese (1987) (as cited in IARC, 1999)
Han rat peripheral lymphocytes	Chromosomal aberrations	+	50 gavage (15 days)	Hayes et al. (2009)
Mouse bone marrow	Chromosomal aberrations	-	15 i.p. × 1	Manna and Das (1973) (as cited in IARC, 1999)
A/L, C57BL/6J mice	Dominant lethal test	+	50 i.p. × 2	Srám et al. (1970) (as cited in IARC, 1999)
ICR/Ha Swiss mice	Dominant lethal test	-	2000 i.p. × 2	Epstein et al. (1972) (as cited in IARC, 1999)
B6C3F1/CRL mice	Sperm morphology	-	2630 i.p. × 5	Wyrobek et al. (1981) (as cited in IARC, 1999)
(CBAxBALB/c)F1 mice	Sperm morphology	In.	1030 i.p. × 5	Topham (1981) (as cited in IARC, 1999)

[a]+, positive; (+), weak positive; - negative; NT, not tested; In., inconclusive.
[b]LED, lowest effective dose; HID, highest ineffective dose; NG, not given; i.p., intraperitoneal.

DERIVATION OF PROVISIONAL VALUES

Table 5A presents a summary of noncancer reference values. Table 5B presents a summary of cancer values for HMPA.

Table 5A. Summary of Noncancer Reference Values for HMPA (CASRN 680-31-9)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Dose	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-day)	Rat/M,F	Increased incidence of nasal lesions	4×10^{-3}	NOAEL	1.2	300	Keller et al. (1997)
Chronic p-RfD (mg/kg-day)	Rat/M,F	Increased incidence of nasal lesions	4×10^{-4}	NOAEL	1.2	3000	Keller et al. (1997)
Subchronic p-RfC (mg/m^3)	N/A						
Chronic p-RfC (mg/m^3)	N/A						

Table 5B. Summary of Cancer Values for HMPA

Toxicity Value	Reference Value	Tumor Type or Precursor Effect	Species/Sex	Principal Study
p-OSF	N/A			
p-IUR	N/A			

N/A = not available

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

Two critical effects were observed in HMPA oral studies conducted with rats: nasal respiratory lesions and testicular atrophy, with the latter effect occurring at higher doses. In addition, HMPA did not cause adverse reproductive or developmental effects in either sex at doses lower than those that caused nasal lesions. The reduction in the fertility index observed in the Shott et al. (1971) reproductive study was not considered a compound-related effect by the study authors because of pneumonia in the rats. Therefore, an increased incidence of nasal

lesions in treated rats (both sexes) observed by Keller et al. (1997) is selected as the critical effect and principal study, respectively, for derivation of the subchronic p-RfD.

The study by Keller et al. (1997) is presented in a peer-reviewed journal and appears appropriate with respect to study design and performance, number of animals, examination of potential toxicity endpoints, and presentation of information. However, it is not stated whether GLP guidelines were followed in this study. Details are provided in the Review of Potentially Relevant Data section. BMD analysis is not possible with the study data because the severity of the nasal lesions was characterized by a qualitative scoring scheme (normal, minimal, mild, marked, or severe) rather than with quantitative incidence data that can be modeled. Therefore, the NOAEL/LOAEL approach is used to determine the point of departure (POD) for HMPA.

Among the available acceptable studies that examined nasal or respiratory lesions as a critical endpoint (see Table 6), the Keller et al. (1997) study represents the lowest credible POD for deriving a subchronic p-RfD. The study authors identified a NOAEL of 1.2 mg/kg-day in male rats. The other two available subchronic-duration studies were not selected as the principal study for the following reasons: relatively high doses were administered; no NOAEL or LOAEL could be identified in Kimbrough and Sedlak (1968); the data reporting for the critical endpoint (nasal/respiratory effects) was poor in the Shott et al. (1971) subchronic-duration study, even though the doses were similar to Keller et al. (1997).

Adjust for daily exposure:

$NOAEL_{ADJ}$ = NOAEL × [conversion to daily dose]
= 1.2 mg/kg × (days dosed ÷ 7 days in week)
= 1.2 mg/kg × (7 ÷ 7)
= 1.2 mg/kg-day × 1
= 1.2 mg/kg-day

A subchronic p-RfD is developed as follows:

Subchronic p-RfD = $NOAEL_{ADJ} \div UF_c$
= 1.2 mg/kg-day ÷ 300
= **4×10^{-3} mg/kg-day**

Table 6. Summary of Relevant Oral Systemic Toxicity Studies for HMPA that Examined Nasal Lesions as a Critical Endpoint

References	# /Sex (M/F)	Exposure (mg/kg-day)	Frequency/ Duration	$NOAEL_{ADJ}$[a] (mg/kg-day)	$LOAEL_{ADJ}$[b] (mg/kg-day)	Critical Endpoint
Keller et al. (1997)	10/10 rat	0, 1.2, 15, 42, 123 (m); 0, 2.3, 20, 63, 229 (f)	7 d/wk for 90-d drinking water	1.2 (m)	15 (m)	**Increase in nasal and tracheal lesions and severity**
Keller et al. (1997)	10/0 rat	0, 15, 40, 120	7 d/wk for 90-d gavage	-[c]	15	**Increase in nasal lesions**
Kimbrough and Sedlak (1968)	20/0 rat	0, 106, 127	# d/wk not reported for 52−72-d dietary	-	-	**Increase in lung lesions, increase in lung weights**
Shott et al. (1971)	20/20 rat	0, 2, 10, 50	7 d/wk for 92-d gavage	10	40	**Increase in respiratory disease**
Kimbrough and Gaines (1973)	15/0 rat	0, 0.78, 1.56, 3.12, 6.25	# d/wk not reported for 2-yr dietary	-	-	**Increase in lung disease at all doses**

[a]$NOAEL_{ADJ}$ = NOAEL × (days dosed ÷ 7 days).
[b]$LOAEL_{ADJ}$ = LOAEL × (days dosed ÷ 7 days).
[c]No NOAEL was identified. NOAEL is considered equal to a LOAEL ÷ 10 for screening purposes.

Tables 7 and 8, respectively, summarize the UFs and the confidence descriptor for the subchronic p-RfD for HMPA.

Table 7. Uncertainty Factors for Subchronic p-RfD of HMPA		
UF	**Value**	**Justification**
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the toxicity of HMPA in the nasal cavity.
UF_D	3	Although there is an available developmental study (Kimbrough and Gaines, 1966) and an available two-generation reproductive study (Shott et al., 1971), uncertainties in the data evaluation and experimental protocols exist within these studies, warranting a partial UF_D of 3.
UF_H	10	A UF_H of 10 for is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study (Keller et al., 1997) was utilized as the principal study.
UF_C $\leq$3000	300	

Table 8. Confidence Descriptor for Subchronic p-RfD for HMPA		
Confidence Categories	**Designation[a]**	**Discussion**
Confidence in Study	**M**	Confidence in the principal study (Keller et al., 1997) is medium because an adequate number of animals were used and experimental protocols were adequately designed, conducted, and reported. The study reported nasal/respiratory pathological effects within a dose range in which a LOAEL and NOAEL could be identified for the critical effect. However, results for the critical endpoint were given qualitatively and could not be modeled.
Confidence in Database	**L**	Confidence in the database was rated low because the results for the critical endpoint were given qualitatively with no information about incidence, and the database lacks studies in species besides rats and lacks adequate reproductive and developmental studies.
Confidence in Subchronic p-RfD[b]	**L**	The overall confidence in the subchronic p-RfD is low.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than lowest entry in table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

In addition to the studies in Table 6 that were evaluated for use in the derivation of the subchronic p-RfD, there is one chronic study (Kimbrough and Gaines, 1973) that was considered. Kimbrough and Gaines (1973) was reported in limited detail. Although pathological examinations were performed on all treated animals at the end of the study, no statistical analyses were done by the study authors. To further examine these results, a dose trend analysis was performed for this review using linear regression analysis and the results are provided in Table 9. The increase in chronic kidney disease at the higher dose levels is not statistically significant. The study authors stated that this incidence of kidney disease did not follow any particular pattern, and, therefore, this effect was not considered chemical-specific. The increase in incidence of lung disease observed at the lower dose levels had a *p*-value of 0.0524. Although this result is marginally statistically significant, it supports the results from the subchronic-duration Keller et al. (1997) study that was chosen for the derivation of the subchronic p-RfD, and it indicates that similar results are found at a similar dose administered chronically. Therefore, Keller et al (1997) was also chosen as the principal study for the derivation of the chronic p-RfD.

Table 9. Trend Analysis Results on Kimbrough and Gaines (1973) Data

	Dose (mg/kg-day)							
	Males				**Females**			
	0	**3.12**	**6.25**	**Trend *p*-value**	**0**	**0.78**	**1.56**	**Trend *p*-value**
No. rats examined	15	15	14	0.3327	15	15	15	0.333
No. rats died	9	12	9	1	10	7	10	1
Malignant tumors	2	-	2	0.999	2	1	2	1
Lung disease	8	12	12	0.334	4	8	11	0.0524
Chronic kidney disease	9	11	12	0.122	13	14	12	0.6667
Testicular atrophy	4	2	6	0.666	3	4	10	0.249

The chronic p-RfD is based on a NOAEL of 1.2 mg/kg-day for nasal respiratory lesions in male rats exposed to HMPA in drinking water for 92 days (Keller et al., 1997). The chronic p-RfD for HMPA is derived as follows:

$$\textbf{Chronic p-RfD} = NOAEL_{ADJ} \div UF_C$$
$$= 1.2 \text{ mg/kg-day} \div 3000$$
$$= \mathbf{4 \times 10^{-4} \text{ mg/kg-day}}$$

Table 10 summarizes the UFs for the chronic p-RfD for HMPA.

Table 10. Uncertainty Factors for Chronic p-RfD of HMPA		
UF	**Value**	**Justification**
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the toxicity of HMPA in the nasal cavity.
UF_D	3	Although there is an available developmental study (Kimbrough and Gaines, 1966) and an available two-generation reproductive study (Shott et al., 1971), uncertainties in the data evaluation and experimental protocols exist within these studies, warranting a partial UF_D of 3.
UF_H	10	A UF_H of 10 for is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	10	A UF_S of 10 is applied for using data from a subchronic-duration study to assess potential effects from chronic-duration exposure because data for evaluating the response from chronic-duration exposure are insufficient.
UF_C	3000	

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

Subchronic-duration toxicity studies for inhaled HMPA are not available.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

Noncancer chronic-duration toxicity studies for inhaled HMPA are not available.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *"Suggestive Evidence of Carcinogenic Potential"* for HMPA by the inhalation route of exposure (see Table 11). This characterization is based on the following findings: (1) chronic-duration inhalation exposure of Charles River rats to low doses of HMPA induced statistically significant increased incidences of squamous-cell carcinomas of the nasal cavity in both sexes (nasal tumors were observed at 400 ppb after 7 months and at 50 ppb after 12 months) (Lee and Trochimowicz, 1982b) and (2) positive results from certain mutagenicity tests provide supporting evidence for the carcinogenic potential of HMPA. As discussed in Section B and tabulated in Tables 3 and 4, the genotoxicity (e.g., clastogenicity, mutagenicity) of HMPA has been extensively studied with mixed results. HMPA was positive in several in vivo mutagenicity assays including the mouse bone marrow micronucleus and the dominant lethal test. HMPA was genotoxic in the *Drosophila melanogaster* sex-linked recessive lethal assay, in the mouse lymphoma assay, and in human hepatoma HepG2 cells. HMPA indicated mixed results for sister chromatid exchange in Chinese hamster ovary cells, both with and without metabolic activation, and it was negative in human lymphocytes in the micronucleus test and for chromosomal aberrations (IARC, 1999).

Animal data evaluating the carcinogenicity of HMPA administered orally are limited, but in a study in rats, no significant increase in tumors was noted after chronic-duration exposure (Kimbrough and Gaines, 1973).

Table 11 identifies the cancer weight-of-evidence descriptor for HMPA.

Table 11. Cancer WOE Descriptor for HMPA

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	Not Selected	Not Applicable	Not Applicable
"Likely to Be Carcinogenic to Humans"	Not Selected	Not Applicable	Not Applicable
"Suggestive Evidence of Carcinogenic Potential"	**Selected**	**Inhalation**	There is one inhalation study showing increased incidence of squamous-cell carcinomas of the nasal cavity in both sexes of rat (Lee and Trochimowicz, 1982a,b,c, 1984). However, a lack of information about the number of rats, either male or female exposed, time of sacrifice, and duration of exposure raises some uncertainties in the cancer classification. Supporting data include in vitro and in vivo genotoxicity/mutagenicity data. There is inadequate information regarding carcinogenicity following oral administration.
"Inadequate Information to Assess Carcinogenic Potential"	Not Selected	Not Applicable	Not Applicable
"Not Likely to Be Carcinogenic to Humans"	Not Selected	Not Applicable	Not Applicable. No strong evidence of noncarcinogenicity in humans or animals is available.

MODE-OF-ACTION DISCUSSION

The HMPA mode of action is still largely unknown. However, studies indicate a role for metabolism of HMPA likely through cytochrome P-450-mediated *N*-demethylation to formaldehyde (HCHO), a rat nasal carcinogen by inhalation. This intracellular release of formaldehyde, together with a mitogenic effect of the compound itself or other metabolites, is suggested to be responsible for HMPA's carcinogenic and mutagenic effects (Bogdanffy et al., 1997; IARC, 1999).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

No p-OSF can be derived due to a lack of carcinogenicity data.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

The effects of chronic-duration inhalation exposure of rats to HMPA were studied by Lee and Trochimowicz (1982a,b,c, 1984). Although the study provides extensive data on the carcinogenic response produced by inhalation of HMPA, the data from this study are not sufficient to support a quantitative cancer dose-response assessment because of uncertainties regarding the experimental design, establishing clear dose-response data (which requires a number of assumptions to be made), and data reporting. For example, it is difficult to determine, based on the published results from this study, how many rats were in each exposure group and at what point in time they were sacrificed. Various tables in the published papers give conflicting accounts of the actual experimental design. The number of animals exposed for each length of time is needed to derive a quantitative risk assessment value for HMPA. In addition, the results from this study are presented in terms of total tumors (both males and females) and are not separated by sex, which also creates uncertainty in terms of deriving a p-IUR from these data. Consideration was given to modeling a subset of the data from Lee and Trochimowicz (1982a,b,c, 1984) to derive a p-IUR. However, as per data presented in the *Chronic-duration Studies* section, there were too many uncertainties in the data for deriving a reasonable value.

APPENDIX A. DATA TABLES

Table A.1. Mean Body Weights of Male Rats Exposed to HMPA in Drinking Water for 90 Days[a]

Test Day	0 mg/kg-day	1.2 mg/kg-day (10 ppm)	15 mg/kg-day (100 ppm)	42 mg/kg-day (300 ppm)	123 mg/kg-day (1000 ppm)
0	302.3 (22.9)[b]	300.4 (19.6)	299.0 (20.6)	298.8 (18.0)	297.6 (17.5)
7	357.4 (25.8)	353.3 (23.7)	354.3 (23.3)	352.0 (25.8)	338.6 (18.6)
15	404.0 (26.8)	395.1 (26.8)	394.5 (26.0)	387.2 (29.8)	375.1 (23.0)[c]
20	433.0 (36.3)	420.3 (28.8)	425.0 (32.4)	414.4 (33.0)	391.4 (23.7)[c]
28	464.0 (43.8)	443.4 (33.6)	451.6 (31.4)	444.5 (35.8)	415.7 (29.5)[c]
35	486.2 (46.4)	467.9 (35.4)	474.1 (34.8)	464.2 (40.3)	431.7 (29.6)[c]
42	517.5 (48.1)	496.7 (36.2)	502.8 (41.2)	491.5 (38.8)	454.4 (33.4)[c]
49	526.7 (41.3)	514.2 (43.4)	517.6 (38.5)	499.2 (47.4)	461.3 (32.4)[c]
56	538.8 (42.5)	527.5 (41.6)	534.8 (51.7)	511.9 (47.6)	474.8 (36.0)[c]
63	565.5 (46.4)	546.2 (49.9)	557.8 (48.5)	530.8 (47.9)	488.3 (37.2)[c]
70	575.1 (43.6)	564.2 (46.10	574.6 (54.3)	544.1 (49.2)	506.8 (35.2)[c]
77	593.5 (51.5)	580.4 (48.0)	592.9 (56.7)	563.7 (52.3)	515.2 (39.9)[c]
84	598.8 (52.0)	582.0 (45.9)	599.2 (61.2)	568.3 (51.7)	522.0 (41.5)[c]
92	600.3 (60.3)	575.3 (54.2)	598.3 (70.9)	552.5 (54.2)	501.3 (38.6)[c]

[a]Keller et al. (1997).
[b]Standard deviation is reported in parentheses.
[c]Statistically significant difference from control ($p < 0.05$).

Table A.2. Mean Relative Organ Weights of Male Rats Exposed to HMPA in Drinking Water for 90 Days[a]

	Dose				
	0 mg/kg-day	**1.2 mg/kg-day (10 ppm)**	**15 mg/kg-day (100 ppm)**	**42 mg/kg-day (300 ppm)**	**123 mg/kg-day (1000 ppm)**
Liver	2.64 ± 0.19	2.68 ± 0.35	2.69 ± 0.25	2.80 ± 0.31	2.83 ± 0.26
Kidneys	0.62 ± 0.05	0.65 ± 0.05	0.63 ± 0.06	0.68 ± 0.05	0.72 ± 0.06[b]
Heart	0.29 ± 0.03	0.30 ± 0.04	0.30 ± 0.04	0.31 ± 0.03	0.32 ± 0.02
Spleen	0.15 ± 0.02	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.05	0.15 ± 0.03
Brain	0.36 ± 0.03	0.37 ± 0.05	0.37 ± 0.04	0.39 ± 0.03	0.42 ± 0.03[b]
Testes	0.59 ± 0.08	0.59 ± 0.12	0.59 ± 0.06	0.61 ± 0.11	0.40 ± 0.15[b]

[a]Keller et al. (1997).
[b]Statistically significant difference from control ($p < 0.05$).

Table A.3. Severity of Respiratory Tract Lesions in Rats Administered HMPA in Drinking Water and Gavage for Approximately 90 Days[a]

	Dose					
	0 mg/kg-day	**1.2 mg/kg-day (10 ppm)**	**15 mg/kg-day (100 ppm)**	**42 mg/kg-day (300 ppm)**	**123 mg/kg-day (1000 ppm)**	**120 mg/kg-day[b] (gavage)**
Trachea (epithelial denudation/regeneration)	normal	minimal	marked	severe	severe	no data
Bronchi (epithelial denudation/regeneration)	normal	normal	minimal	mild	marked	no data
Nose (epithelial denudation/inflammation, Level I, II)	normal	normal	mild	mild	mild	mild
Nose (epithelial denudation/inflammation, Level III, IV)	normal	normal	minimal	marked	severe	severe
Nose (adhesion, nasal turbinates/septum, Level I, II)	normal	normal	normal	mild	severe	marked
Nose (adhesion, ethmoturbinates, Level III, IV)	normal	normal	normal	mild	severe	severe
Nose (epithelial regeneration/sq. metaplasia, Level I, II)	normal	normal	mild	mild	mild	mild
Nose (nasoturbinate bone proliferation, Level I, II)	normal	normal	normal	minimal	mild	mild
Nose (ethmoturbinate bone proliferation, Level III, IV)	normal	normal	minimal	mild	severe	severe

[a]Keller et al. (1997).
[b]The gavage exposure was included in the table to compare with the drinking water exposures.

Table A.4. Severity of Respiratory Tract Lesions in Male Rats Administered HMPA by Gavage and Implant for Approximately 90 Days[a]

	Dose				
	0	**15 mg/kg-day**	**40 mg/kg-day**	**120 mg/kg-day**	**40 mg/kg-day (Implant)**
Trachea (epithelial denudation/regeneration)	NA	NA	NA	NA	NA
Bronchi (epithelial denudation/regeneration)	NA	NA	NA	NA	NA
Nose (epithelial denudation/inflammation, Level I, II)	normal	mild	marked	mild	mild
Nose (epithelial denudation/inflammation, Level III, IV)	normal	marked	marked	severe	marked
Nose (adhesion, nasal turbinates/ septum, Level I, II)	normal	normal	mild	marked	mild
Nose (adhesion, ethmoturbinates, Level III, IV)	normal	normal	marked	severe	normal
Nose (epithelial regeneration/sq. metaplasia, Level I, II)	normal	normal	mild	mild	mild
Nose (nasoturbinate bone proliferation, Level I, II)	normal	normal	minimal	mild	mild
Nose (ethmoturbinate bone proliferation, Level III, IV)	normal	normal	marked	severe	mild

[a]Keller et al. (1997).

Table A.5. Pathology Findings of Male Rats Fed HMPA for 2 Years[a]

	Concentration					
	0 mg/kg-day (Part 1)	**0 mg/kg-day (Part 2)**	**0.78 mg/kg-day (Part 2)**	**1.56 mg/kg-day (Part 2)**	**3.12 mg/kg-day (Part 1)**	**6.25 mg/kg-day (Part 1)**
Number of male rats examined	15	15	15	15	15	14
Number of male rats died	9	10	7	10	12	9
Malignant tumors	2	2	1	2	-	2
Lung disease	8	4	8	11	12	12
Chronic kidney disease	9	13	14	12	11	12
Testicular atrophy	4	3	4	10	2	6

[a]Kimbrough and Gaines (1973).

Table A.6. Reproduction Indices of Rats Exposed to HMPA by Gavage[a]

Dose (mg/kg-day)	Generation P1 $F1_A$				Generation P1 $F1_B$				Generation P1 $F1_C$				Generation P2 $F2_A$			
	FI	**GI**	**LBI**	**LI**	**FI**	**GI**	**LBI**	**LI**	**FI**	**GI**	**LBI**	**LI**	**FI**	**GI**	**LBI**	**LI**
0	85	100	99.5	87.4	89.5	94.1	99.5	79.7	82.4	85.7	88.8	77.8	95	100	100	94
2	90	100	96.1	88.8	60	88.8	100	70.3	63.6	100	100	87.5	95	100	100	98
10	85	100	99.5	87.4	47.1	87.5	98.6	87.5	76.9	90	100	97.2	100	100	100	98.6

[a]Shott et al. (1971).

FI = fertility index; GI = gestation index; LBI = live birth index; LI = lactation index.

Table A.7. Incidence of Pneumonia and Testicular Atrophy in Male Rats Exposed to HMPA by Gavage[a]

	Concentration			
	0 mg/kg-day	**500 mg/kg-day**	**1000 mg/kg-day**	**2000 mg/kg-day**
Number of rats examined	6	6	5	9
Number of rats sacrificed	6	6	5	9
Number of rats died	0	0	0	0
Number of rats with pneumonia	0	1	3	2
Number of rats with partial or complete testicular atrophy	0	0	2	9

[a]Kimbrough and Gaines (1966).

Table A.8. Incidence of Pneumonia and Testicular Atrophy in Male Rats Exposed to HMPA by Gavage[a]

	Concentration			
	0 mg/kg-day	**100 mg/kg-day**	**200 mg/kg-day**	**400 mg/kg-day**
Number of rats examined	10	10	5	8
Number of rats sacrificed	10	9	3	2
Number of rats died	0	1	2	6
Number of rats with pneumonia	0	5	3	2
Number of rats with partial or complete testicular atrophy	0	6	4	5

[a]Kimbrough and Gaines (1966).

Table A.9. Exposure Protocol Adapted from Lee and Trochimowicz, 1984							
Group	**# Animals (M&F)**	**Dose (ppb)**	**Exposure Time (months)**	**Number Sacrificed at each Time Interval (months)**			
				3	**8**	**12**	**24**
1, 2	240	0	24	18	-	20	R
1, 2A*	200	0	24	-	-	-	R
9, 10*	200	10	24	-	-	-	R
3, 4	120	50	12	-	-	-	R
3, 4	120	50	24	18	6	20	R
3, 4A*	200	50	24	-	-	20	R
11, 12*	100	100	6	-	-	-	R
11, 12*	100	100	13	-	-	-	R
5, 6	240	400	10	18	-	-	R
7, 8	240	4000	9	18	-	-	R

*Groups belonging to the second experiment.

Groups with odd numbers are males, and those with even numbers are females.
R = all remaining animals.

Table A.10. Incidence of Nasal Tumors in Rats Exposed to HMPA for Up to 24 Months[a]

	Concentration					
	0	**10 ppb**	**50 ppb**	**100 ppb**	**400 ppb**	**4000 ppb**
Number of rats examined	396	200	194	200	219	215
Papilloma	0	0	9 (4.6%)	6 (3.0%)	13 (5.9%)	11 (5.1%)
Adenomatoid polyp	0	0	0	1	0	0
Epidermoid carcinoma	0	0	24 (12.4%)	59 (29.5%)	137 (62.6%)	120 (55.8%)
Adenoid squamous carcinoma	0	0	4 (2.1%)	5 (2.5%)	21 (9.6%)	41 (19.1%)
Adenocarcinoma	0	0	1 (0.5%)	1 (0.5%)	2 (0.9%)	2 (0.9%)
Transitional carcinoma	0	0	1 (0.5%)	1 (0.5%)	3 (1.4%)	4 (1.9%)
Undifferentiated carcinoma	0	0	0	2 (1.0%)	2 (0.9%)	1 (0.5%)
Pleomorphic (mixed) tumor	0	0	0	0	2 (0.9%)	0
Total tumor/grp			39/194 (20.0%)	75/200 (37.5%)	180/219 (82.2%)	179/215 (83.3%)

[a]Lee and Trochimowicz (1982b).

APPENDIX B. BMD OUTPUTS

Appendix B is not applicable.

APPENDIX C. REFERENCES

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