United States Environmental Protection Agency

EPA/690/R-20/005F | September 2020 | FINAL

Provisional Peer-Reviewed Toxicity Values for

N,N,N′,N″-Tetramethylphosphoramide (TMPA) (CASRN 16853-36-4)

U.S. EPA Office of Research and Development
Center for Public Health and Environmental Assessment

EPA/690/R-20/005F
September 2020
https://www.epa.gov/pprtv

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(CASRN 16853-36-4)

Center for Public Health and Environmental Assessment
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR N,N,N′,N″-TETRAMETHYLPHOSPHORAMIDE (TMPA) (CASRN 16853-36-4)

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.

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.

Currently available PPRTV assessments can be accessed on the U.S. Environmental Protection Agency's (EPA's) PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing adverse human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

QUALITY ASSURANCE

This work was conducted under the U.S. EPA Quality Assurance (QA) program to ensure data are of known and acceptable quality to support their intended use. Surveillance of the work by the assessment managers and programmatic scientific leads ensured adherence to QA processes and criteria, as well as quick and effective resolution of any problems. The QA manager, assessment managers, and programmatic scientific leads have determined under the QA program that this work meets all U.S. EPA quality requirements. This PPRTV was written with guidance from the CPHEA Program Quality Assurance Project Plan (PQAPP), the QAPP titled *Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents (L-CPAD-0032718-QP)*, and the PPRTV development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP)*. As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

All PPRTV assessments receive internal peer review by at least two Center for Public Health and Environmental Assessment (CPHEA) scientists and an independent external peer review by at least three scientific experts. The reviews focus on whether all studies have been correctly selected, interpreted, and adequately described for the purposes of deriving a provisional reference value. The reviews also cover quantitative and qualitative aspects of the provisional value development and address whether uncertainties associated with the assessment have been adequately characterized.

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. 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.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVS

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) CPHEA website at https://www.epa.gov/pprtv/forms/contact-us-about-pprtvs.

INTRODUCTION

N,N,N′,N″-Tetramethylphosphoramide (TMPA), CASRN 16853-36-4, belongs to the class of compounds known as phosphoramides. It is used as a solvent (Yang et al., 2011). TMPA is not listed on U.S. EPA's Toxic Substances Control Act's public inventory (U.S. EPA, 2018b), nor is it registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2018).

The empirical formula for TMPA is $C_4H_{14}N_3OP$, and its structure is shown in Figure 1. Table 1 summarizes the physicochemical properties of TMPA. TMPA is a liquid at room temperature. TMPA's moderate vapor pressure and Henry's law constant indicate that it is not expected to volatilize from either dry or moist surfaces. If TMPA does partition to the atmosphere, the estimated vapor pressure indicates that it will exist there almost entirely as a vapor. The estimated half-life of vapor-phase TMPA in air by reaction with photochemically produced hydroxyl radicals is 1.5 hours. The estimated high water solubility, and low soil adsorption coefficient for TMPA indicate that it may leach to groundwater or undergo runoff after a rain event.

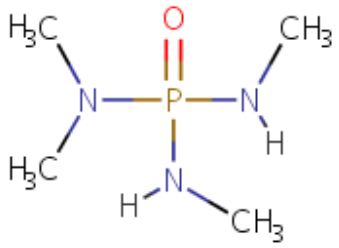


Figure 1. N,N,N′,N″-Tetramethylphosphoramide (CASRN 16853-36-4) Structure

Table 1. Physicochemical Properties of TMPA (CASRN 16853-36-4)[a]	
Property (unit)	**Value[a]**
Physical state	Liquid
Boiling point (°C)	226 (predicted average)
Melting point (°C)	69.4 (predicted average)
Density (g/cm^3)	1.12 (predicted average)
Vapor pressure (mm Hg at 25°C)	0.243 (predicted average)
pH (unitless)	NV
pKa (unitless)	NV
Water solubility (mol/L)	4.44 (predicted average)
Octanol-water partition coefficient (log *P*)	−1.14 (predicted average)
Henry's law constant (atm-m^3/mol at 25°C)	8.75×10^{-8} (predicted average)
Soil adsorption coefficient K_{oc} (L/kg)	20.8 (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	4.67×10^{-11} (predicted average)
Atmospheric half-life (hours)	NV
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	151.15
Flash point (°C)	67.5 (predicted average)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (N,N,N',N″-tetramethylphosphoramide CASRN 16853-36-4; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID80168576; accessed April 20, 2020). All values are experimental averages unless otherwise specified.

NA = not applicable; NV = not available; TMPA = N,N,N′,N″-tetramethylphosphoramide.

There are no available toxicity values for TMPA from U.S. EPA or other agencies/organizations (see Table 2).

Table 2. Summary of Available Toxicity Values for TMPA (CASRN 16853-36-4)			
Source[a]	**Value**	**Notes**	**Reference(s)[b]**
Noncancer			
IRIS	NV	NA	U.S. EPA (2018a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2012a)
ATSDR	NV	NA	ATSDR (2017)
IPCS	NV	NA	IPCS (2018)
CalEPA	NV	NA	CalEPA (2016); CalEPA (2018a); CalEPA (2018b)
OSHA	NV	NA	OSHA (2017b); OSHA (2017a)
NIOSH	NV	NA	NIOSH (2016)
ACGIH	NV	NA	ACGIH (2017)
Cancer			
IRIS	NV	NA	U.S. EPA (2018a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2012a)
NTP	NV	NA	NTP (2016)
IARC	NV	NA	IARC (2018)
CalEPA	NV	NA	CalEPA (2011); CalEPA (2018a); CalEPA (2018b)
ACGIH	NV	NA	ACGIH (2017)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.
[b]Reference date is the publication date for the database and not the date the source was accessed.

NA = not applicable; NV = not available; TMPA = N,N,N′,N″-tetramethylphosphoramide.

Non-date-limited literature searches were conducted in March 2018 and updated in January 2020 for studies relevant to the derivation of provisional toxicity values for TMPA (CASRN 16853-36-4). The database searches for PubMed, TOXLINE (including TSCATS1), and Web of Science were conducted by an information specialist and records stored in the U.S. EPA's Health and Environmental Research Online (HERO) database. The following additional databases were searched for health-related data: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), U.S. EPA TSCATS2/TSCATS8e, U.S. EPA High Production Volume Information System (HPVIS), International Agency for Research on Cancer (IARC), International Programme on Chemical Safety (IPCS)/INCHEM, Japan Existing Chemical Data Base (JECDB), National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS), International Uniform Chemical Information Database (IUCLID), High Production Volume (HPV), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

As shown in Tables 3A and 3B, there are no potentially relevant short-term, subchronic, or chronic studies or developmental or reproductive toxicity studies in humans or animals. The phrase "statistical significance" or the term "significant," used throughout the document, indicates a p-value of < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for TMPA (CASRN 16853-36-4)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data; TMPA = N,N,N′,N″-tetramethylphosphoramide.

Table 3B. Summary of Potentially Relevant Cancer Data for TMPA (CASRN 16853-36-4)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry**	**Critical Effects**	**Reference**	**Notes**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data; TMPA = N,N,N′,N″-tetramethylphosphoramide.

HUMAN STUDIES

No human exposure studies have been identified.

ANIMAL STUDIES

No animal exposure studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Toxicokinetics

There are no toxicokinetic data for TMPA following oral or inhalation exposure. However, some metabolic and excretion information can be inferred from studies of related phosphoramides, including hexamethylphosphoramide (HMPA), and pentamethylphosphoramide (PMPA). HMPA, the parent chemical to TMPA, is broken down into lower methylphosphoramides via sequential oxidative demethylation by cytochrome P450s (CYP450s), yielding PMPA, TMPA, and N,N′,N″-trimethylphosphoramide (triMPA). At each demethylation step, the reaction forms unstable methylol intermediates that break down into the demethylated phosphoramides, releasing formaldehyde in the process (Jones, 1970; Jones and Jackson, 1968). A minor metabolic pathway for HMPA and PMPA involves the formation of *N*-formyl-PMPA and *N*-formyl-TMPA, respectively, from the unstable methylol intermediates (Jones, 1970). The toxicological consequences of products generated through this minor metabolic pathway is currently unclear. A schematic of the proposed metabolism of TMPA can be found in Appendix A (see Figure A-1).

Genotoxicity

The genotoxicity data for TMPA are limited to a single study using an unknown isomer of TMPA at doses up to 5 mM. The study reported no induction of mitotic recombination in the *Drosophila melanogaster* white/white$^+$ (w/w$^+$) eye mosaic system (Vogel and Nivard, 1993).

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF ORAL REFERENCE DOSES

No studies have been located regarding toxicity of TMPA to humans or animals by oral exposure; therefore, subchronic and chronic provisional reference doses (p-RfDs) were not derived directly. Instead, screening subchronic and chronic p-RfDs are developed in Appendix A using an alternative analogue surrogate approach. Based on the overall analogue approach presented in Appendix A, HMPA was selected as the most appropriate analogue for TMPA for deriving a screening subchronic and chronic p-RfD (see Table 4).

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies have been located regarding toxicity of TMPA to humans or animals by inhalation; therefore, subchronic and chronic provisional reference concentrations (p-RfCs) were not derived directly. An alternative analogue approach was not attempted for the p-RfCs because of limitations in the inhalation toxicity data for the candidate analogue (see Appendix A).

Table 4. Summary of Noncancer Reference Values for TMPA (CASRN 16853-36-4)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rats/M	Increased incidence of nasal lesions	1×10^{-3}	NOAEL	0.29 (based on analogue POD)	300	Keller et al. (1997) as cited in U.S. EPA (2012b)
Screening chronic p-RfD (mg/kg-d)	Rats/M	Increased incidence of nasal lesions	1×10^{-4}	NOAEL	0.29 (based on analogue POD)	3,000	Keller et al. (1997) as cited in U.S. EPA (2012b)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

HED = human equivalent dose; M = male(s); NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; TMPA = N,N,N′,N″-tetramethylphosphoramide; UF_C = composite uncertainty factor.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is *"Inadequate Information to Assess the Carcinogenic Potential"* of TMPA (see Table 5). No relevant studies are available in humans or animals. Within the current U.S. EPA Cancer Guidelines (U.S. EPA, 2005), there is no standard methodology to support the identification of a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates for data-poor chemicals using an analogue approach. In the absence of an established framework, a screening evaluation of potential carcinogenicity is provided using the methodology described in Appendix B. A screening evaluation of potential carcinogenicity is provided using methodology described in

Appendix B. This evaluation determined that there was a qualitative level of *concern for potential carcinogenicity* for TMPA (see Appendix C).

Table 5. Cancer WOE Descriptor for TMPA (CASRN 16853-36-4)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human carcinogenicity data identified to support this descriptor.
"Likely to Be Carcinogenic to Humans"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**This descriptor is selected due to the lack of adequate data in humans or animals to evaluate the carcinogenic potential of TMPA.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No evidence of noncarcinogenicity is available.

NA = not applicable; NS = not selected; TMPA = N,N,N′,N″-tetramethylphosphoramide; WOE = weight of evidence.

DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

The absence of suitable data precludes development of cancer risk estimates for TMPA (see Table 6).

Table 6. Summary of Cancer Risk Estimates for TMPA (CASRN 16853-36-4)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Risk Estimate	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor; TMPA = N,N,N′,N″-tetramethylphosphoramide.

APPENDIX A. SCREENING NONCANCER PROVISIONAL VALUES

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional toxicity values for N,N,N′,N″-tetramethylphosphoramide (TMPA). However, information is available for this chemical, which although insufficient to support deriving a provisional toxicity value under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the provisional reference values to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there could be more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the CPHEA.

APPLICATION OF AN ALTERNATIVE ANALOGUE APPROACH

The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012). Three types of potential analogues (structural, metabolic, and toxicity-like) are identified to help select the final analogue chemical. The analogue approach may or may not be route specific or applicable to multiple routes of exposure. All information was considered together as part of the final weight-of-evidence (WOE) approach to select the most suitable analogue both toxicologically and chemically.

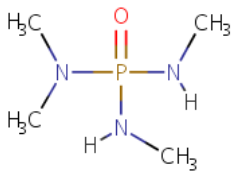
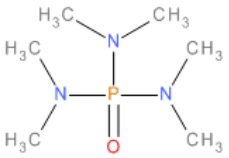
Structural Analogues

An initial analogue search focused on identifying structurally similar chemicals with toxicity values from the Integrated Risk Information System (IRIS), PPRTV, Agency for Toxic Substances and Disease Registry (ATSDR), or California Environmental Protection Agency (CalEPA) databases to take advantage of the well-characterized chemical-class information. Under Wang et al. (2012), structural similarity for analogues is typically evaluated using U.S. EPA's DSSTox database and the National Library of Medicine's (NLM's) ChemIDplus database (ChemIDplus, 2016). Additionally, the Organisation for Economic Co-operation and Development (OECD) Toolbox was used to calculate structural similarity using the Tanimoto method (a quantitative method shared with ChemIDplus) (OECD, 2018).

Upon expert review of the structural fragments contained within the target chemical (TMPA), several phosphoramides were identified as potential structural analogues. Hexamethylphosphoramide (HMPA), pentamethylphosphoramide (PMPA), and N,N′,N″-trimethylphosphoramide (triMPA) were identified as relevant toxicokinetic precursors or metabolites of the target compound because they share sufficient structural similarity. Of these, only HMPA was associated with a published toxicity value. As such, HMPA was the only structural analogue to TMPA that was further considered (U.S. EPA, 2012b). Table A-1 summarizes the analogue's physicochemical properties and similarity scores. ChemIDplus and OECD Toolbox similarity scores for HMPA were 77 and 29%, respectively. TMPA and HMPA are phosphoric acid amide derivatives, differing by two methyl substitutions. Physicochemical

properties (i.e., low octanol-water partition coefficient) for TMPA and HMPA suggest that both of these compounds will be bioavailable following oral and inhalation exposure (see Table A-1). Lastly, while not available for TMPA, a low pKa reported for HMPA suggests that HMPA may be an irritant or corrosive (see Table A-1). This characteristic may promote portal-of-entry effects. In total, HMPA is considered an appropriate structural analogue for TMPA based on commonalities in structural and physicochemical properties.

Table A-1. Physicochemical Properties of TMPA (CASRN 16853-36-4) and its Candidate Analogue[a]

Chemical	**TMPA**	**HMPA**
Structure		
CASRN	16853-36-4	680-31-9
Molecular weight (g/mol)	151.15	179.204
OECD Toolbox similarity score (%)[b]	100	29
ChemIDplus similarity score (%)[c]	100	77
Melting point (°C)	69.4 (predicted average)	6.95
Boiling point (°C)	226 (predicted average)	233
Vapor pressure (mm Hg at 25°C)	0.243 (predicted average)	0.0460
Henry's law constant (atm-m^3/mol at 25°C)	8.75×10^{-8} (predicted average)	8.86×10^{-4} (predicted average)
Water solubility (mol/L)	4.44 (predicted average)	1.87 (predicted average)
Octanol-water partition coefficient (log *P*)	−1.14 (predicted average)	0.195 (predicted average)
pKa	NA	<1.6[b]

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (N,N,N',N"-tetramethylphosphoramide, CASRN 16853-36-4; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID80168576; and hexamethylphosphoramide, CASRN 680-31-9; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID6020694#properties; accessed April 20, 2020). All values are experimental averages unless otherwise specified.
[b]OECD (2018).
[c]ChemIDplus Advanced, similarity scores (ChemIDplus, 2018).

HMPA = hexamethylphosphoramide; NV = not available; OECD = Organisation for Economic Co-operation and Development; TMPA = N,N,N′,N″-tetramethylphosphoramide.

Metabolic Analogues

Table A-2 summarizes available toxicokinetic data for TMPA and its structurally similar analogue, HMPA. As discussed in the "Toxicokinetics" section in the main document, there are no toxicokinetic data following oral or inhalation exposure to TMPA; however, some metabolic and excretion information can be inferred from studies of related phosphoramides, including its parent compound and candidate analogue, HMPA. The induction of toxic responses with oral

and inhalation administration of HMPA provides evidence for its absorption (U.S. EPA, 2012b), and additional studies directly demonstrate that HMPA is well absorbed and distributed throughout the body after both oral and inhalation administration. HMPA exhibits preferential deposition in rat nasal tissue, a major target organ of toxicity for this chemical [Rickard and Guilles (1982) as cited in Keller et al. (1997)].

TMPA is a primary intermediate in the metabolism of HMPA and results from sequential demethylation of the parent compound by cytochrome P450 (CYP450) (Dahl and Brezinski, 1985; Jones and Jackson, 1968). Formaldehyde is released from methylol intermediates at each demethylation step (see Figure A-1) (U.S. EPA, 2012b; Jones and Jackson, 1968). These unstable methylol intermediates of HMPA, PMPA, and TMPA can form minor metabolites, including *N*-formyl-PMPA and *N*-formyl-TMPA, respectively (Jones, 1970). TMPA was readily detected in rat urine following gavage administration of HMPA or PMPA, and formation of its demethylated product, triMPA, has been also demonstrated in animals (Jones and Jackson, 1968). Rapid elimination of the parent phosphoramide, HMPA (>90% elimination within 24 hours after inhalation exposure), has been reported in experimental animals [Rickard and Quarles (1981) as cited in Keller et al. (1997)].

Together, these findings provide support for the in vivo oxidative demethylation of TMPA and suggest that TMPA could share a similar metabolic fate as HMPA in the body. Furthermore, toxicokinetics data showed that HMPA is well absorbed and distributed throughout the body after both oral and inhalation treatments, displaying preferential deposition in rat nasal tissue, a major target organ of toxicity for this chemical [Rickard and Gillies (1982) as cited in Keller et al. (1997)]. While it is obvious that inhalation exposure can promote nasal deposition of inhaled chemicals, Rickard and Guilles (1982) used ^{14}C-HMPA gavage exposure to demonstrate that the naso-, ethmo-, and maxilloturbinate regions of rat nasal passages retained 3- to 7-fold more radioactivity (compared with 7-, 21-, and 25-fold increases after inhalation exposure) than other tissues in the body.

Table A-2. ADME Data for TMPA (CASRN 16853-36-4) and Its Candidate Analogue

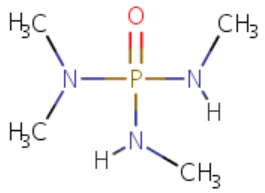
Type of Data	TMPA	HMPA
Structure		
CASRN	16853-36-4	680-31-9
Absorption		
Rate and extent of oral absorption	ND	ND
Rate and extent of inhalation absorption	ND	ND

Table A-2. ADME Data for TMPA (CASRN 16853-36-4) and Its Candidate Analogue		
Type of Data	**TMPA**	**HMPA**
Distribution		
Extent of distribution	ND	Generally widespread distribution with evidence of increased deposition of HMPA and its metabolites (no information on specific metabolites was provided) in nasal tissue following oral or inhalation exposures [Rickard and Gillies (1982) as cited in Keller et al. (1997)]
Metabolism		
Pathways and enzymes	Oxidative demethylation by CYPs; based on metabolic pathways established following oral administration of HMPA or PMPA (Jones, 1970; Jones and Jackson, 1968)	Oxidative demethylation by CYP in liver, lung, and nasal cavity; CYP2A homologues have been shown to be active in metabolic activation of HMPA in vitro (human CYP2A6, rat CYP2A3, rabbit CYP2A10/11) (Thornton-Manning et al., 1997; Liu et al., 1996; Gervasi et al., 1991; Longo et al., 1988; Dahl and Brezinski, 1985; Jones and Jackson, 1968)
Metabolites	Unstable methylol intermediates break down to form triMPA and formaldehyde; based on metabolic pathways established following oral administration of HMPA or PMPA (see Figure A-1) (U.S. EPA, 2012b; Jones, 1970; Jones and Jackson, 1968)	Primary: Unstable methylol intermediates break down to form demethylated phosphoramides (PMPA, TMPA, triMPA); formaldehyde is released at each demethylation step (see Figure A-1) (U.S. EPA, 2012b; Jones, 1970; Jones and Jackson, 1968) Minor: Unstable methylol intermediates break down to form *N*-formyl-PMPA (Jones, 1970)
Excretion		
Rate of excretion	ND	Excretion is rapid (i.e., 90% excreted within 24 hr following inhalation exposure; “most” excreted within 20 hr of i.p. injection) [Rickard and Gillies (1982) as cited in Keller et al. (1997)]
Route of excretion	Metabolic excretion expected via the urine, based on excretion of TMPA and triMPA as metabolites following oral administration of HMPA or PMPA (Jones, 1970; Jones and Jackson, 1968)	Parent compound and metabolites excreted in urine (>90% administered dose; relative amounts of parent/metabolites not quantified) (Jones, 1970; Jones and Jackson, 1968)

ADME = absorption, distribution, metabolism, and excretion; CYP = cytochrome P;
HMPA = hexamethylphosphoramide; i.p. = intraperitoneal; ND = no data; PMPA = pentamethylphosphoramide;
TMPA = N,N,N′,N″-tetramethylphosphoramide; triMPA = N,N′,N″-trimethylphosphoramide.

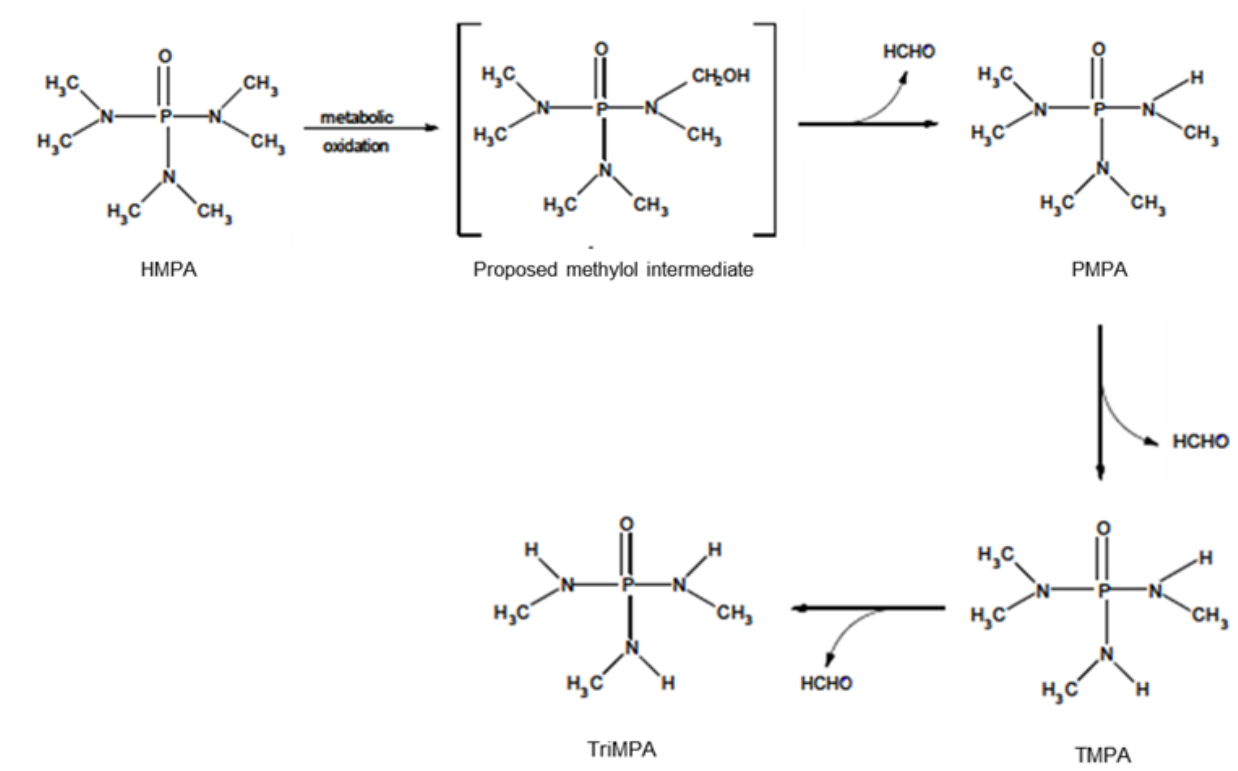


Figure A-1. Primary Pathway of HMPA (CASRN 680-31-9) Metabolism (U.S. EPA, 2012b)

The primary metabolic pathway by which HMPA and TMPA progressively break down into "lower" sequentially demethylated methylphosphoramides (PMPA and triMPA), leading to the production of formaldehyde, is of toxicological relevance, given that both HMPA and formaldehyde are known nasal toxicants in the rat (U.S. EPA, 2012b; Kerns et al., 1983; Swenberg et al., 1980). Based on the data available, it is assumed that the systemic distribution of HMPA and localized (nasal) metabolism is likely to promote the production of formaldehyde (in addition to lower phosphoramides) in nasal tissues following oral exposure to HMPA. Thus, the nasal lesions observed following exposure to HMPA/PMPA/TMPA may be attributable to the localized production of formaldehyde, consistent with the portal-of-entry nasal lesions observed following inhalation exposure to formaldehyde. To this point, microsomal enzymes isolated from the olfactory epithelium of dogs are capable of metabolizing HMPA more efficiently than liver microsomal enzymes (Dahl et al., 1982). Furthermore, the pattern and severity of nasal lesions induced by HMPA after oral exposure coincides with the selective deposition of HMPA and its metabolites (no information on specific metabolites was provided) in the nasal epithelium (Keller et al., 1997). Thus, metabolism is thought to play a role in the respiratory tract toxicity of HMPA following both oral and inhalation exposure (Keller et al., 1997). Because HMPA and TMPA share a similar potential bioactivation pathway, HMPA is considered an appropriate metabolic analogue for TMPA.

Toxicity-Like Analogues

Table A-3 summarizes available oral toxicity values for TMPA and its potential analogue, HMPA. There are no toxicity data for TMPA. Repeated-dose toxicity information for HMPA, the candidate analogue, indicates that the primary targets of toxicity are the respiratory system (particularly the nasal epithelium) and the testes (U.S. EPA, 2012b). The point of departure (POD) for deriving provisional reference doses (p-RfDs) for HMPA is based on a no-observed-adverse-effect level (NOAEL) of 1.2 mg/kg-day for increased incidence of nasal lesions in Sprague-Dawley (S-D) rats (U.S. EPA, 2012b). The principal study used in the HMPA assessment reported dose-related increases in nasal/respiratory tract lesions (epithelial denudation, regeneration, or squamous metaplasia) at doses ≥15 mg/kg-day and testicular effects (testicular atrophy and decreased testes weight) at a dose of 123 mg/kg-day after a 90-day administration of HMPA via drinking water (Keller et al., 1997). Nasal and pulmonary toxicity were also found in other subchronic and chronic studies with gavage or dietary exposure to HMPA at similar doses (see Table A-3) (Kimbrough and Gaines, 1966). Likewise, reproductive studies provided further evidence for the male reproductive effects of HMPA occurring at doses ≥40 mg/kg-day in rats and at a dose of 100 mg/kg-day in rabbits, indicating that these effects are less sensitive than the upper respiratory tract toxicity of HMPA (see Table A-3) (Jackson and Craig, 1966).

Table A-3. Comparison of Available Toxicity Data for TMPA (CASRN 16853-36-4) and Its Candidate Analogue

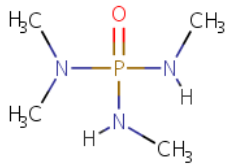
Type of Data	TMPA	HMPA
Structure		
CASRN	16853-36-4	680-31-9
Repeated-dose toxicity—oral subchronic		
Critical effects	NA	Increased incidence of nasal and tracheal lesions at 15 mg/kg-d in males and 20 mg/kg-d in females [Keller et al. (1997) as cited in U.S. EPA (2012b)]
Other effects (in principal study)	NA	Testicular atrophy, reduced absolute and relative testes weight, and reduced body weight in males at 123 mg/kg-d [Keller et al. (1997) as cited in U.S. EPA (2012b)]
Species	NA	Rat
Duration	NA	90 d
Route	NA	Drinking water
Additional toxicity data (from other studies)	ND	Additional data reported in the principal study, nasal and pulmonary lesions have been reported in subchronic studies in rats exposed to HMPA via gavage at ≥15 mg/kg-d or via diet at ~115 mg/kg-d (only dose tested) (Keller et al., 1997). Another toxicity target following short-term- or subchronic-HMPA exposure is the male reproductive system. In rats, testicular atrophy has been observed at ≥40 mg/kg-d (Kimbrough and Gaines, 1966), decreased male fertility at ≥50 mg/kg-d and decreased testicular weight at ≥100 mg/kg-d. Decreased fertility and impaired spermatogenesis has been reported in male rabbits following short-term exposure to 100 mg/kg-d. Fertility and sperm effects following short-term exposure in rats and rabbits were reversible. No adverse testicular effects were reported in mice following short-term exposure to doses up to 500 mg/kg-d (Jackson et al., 1969). No adverse reproductive or developmental effects were observed in a 2-generation gavage study in rats at doses up to 10 mg/kg-d (Shott et al., 1971). No subchronic inhalation data are available.

Table A-3. Comparison of Available Toxicity Data for TMPA (CASRN 16853-36-4) and Its Candidate Analogue

Type of Data	TMPA	HMPA
Repeated-dose toxicity—oral chronic		
Additional toxicity data (from other studies)	NA	A 2-yr dietary study in rats found increased incidence of lung disease at doses ≥0.78 mg/kg-d. The study was inadequate for quantitative risk assessment owing to limited data reporting and lack of statistical analyses (Kimbrough and Gaines, 1973). In an inhalation cancer bioassay (limited by inadequate reporting of study design and results), damage to the nasal tissues and nasal tumors were observed at ≥0.37 mg/m^3 (no other noncancer or cancer effects were reported) [Lee and Trochimowicz (1984, 1982a, 1982b, 1982c) as cited in U.S. EPA (2012b)].

HMPA = hexamethylphosphoramide; NA = not available; TMPA = N,N,N′,N″-tetramethylphosphoramide.

HMPA is also a potent nasal toxicant via the inhalation route, yet provisional reference concentrations (p-RfCs) were not derived because no subchronic inhalation studies were available and the data from the lone chronic inhalation study (Lee and Trochimowicz, 1984, 1982a, b, c) were not sufficient to support a quantitative dose-response assessment. This was due to significant 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. These same uncertainties in experimental design and data reporting in the inhalation bioassay for HMPA further prevented the data from being used to derive provisional cancer potency values for this chemical (U.S. EPA, 2012b).

Metabolic transformation of HMPA to lower methylphosphoramides (including TMPA) and formaldehyde is a proposed mechanism for the critical effects (nasal toxicity) of this compound (U.S. EPA, 2012b; Keller et al., 1997). Because both HMPA and TMPA share this primary metabolic pathway (see "Metabolic Analogues" section and Table A-2 above), the proposed mechanism of action for HMPA-induced nasal toxicity is plausible for TMPA, as well. The testicular effects observed at higher HMPA doses may be related to the number of methyl groups on the parent compound (and subsequent intracellular levels of formaldehyde produced as a product of sequential demethylation), rather than metabolic products, as indicated by reproductive studies in rodents and insects (Jones, 1970; Jackson et al., 1969; Jones and Jackson, 1968). However, the absence of information specific to TMPA prevents an assessment of its potential reproductive effects. While the lack of toxicity data for TMPA precludes the determination of appropriate toxicity-like analogues, the putative bioactivation pathway for nasal toxicity common to TMPA and HMPA indicates that TMPA is a potential respiratory tract toxicant.

Weight-of-Evidence Approach

A WOE approach is used to evaluate information from potential candidate analogues as described by Wang et al. (2012). Commonalities in structural/physicochemical properties, toxicokinetics, metabolism, toxicity, or mode of action (MOA) between potential analogues and chemical(s) of concern are identified. Emphasis is given to toxicological and/or toxicokinetic similarity over structural similarity. Analogue candidates are excluded if they do not have commonality or demonstrate significantly different physicochemical properties and toxicokinetic profiles that set them apart from the pool of potential analogues and/or chemical(s) of concern. From the remaining potential analogues, the most biologically or toxicologically relevant analogue chemical is selected based on MOA information, otherwise, the analogue with the highest structural similarity and/or most conservative toxicity value is selected.

Oral Noncancer

The only candidate compound identified, HMPA, is considered a structural analogue of TMPA based on structural commonalities and physicochemical properties. HMPA is also a parent chemical to TMPA and both compounds are metabolized similarly to lower methylphosphoramides and formaldehyde via a potential bioactivation pathway for nasal toxicity. Thus, HMPA is considered a metabolic analogue for TMPA. Nasal lesions (the critical effect for HMPA) were attributed to systemic distribution of the metabolic product, or the parent compound. Systemic distribution of the parent compound (or any of the expected methylol intermediates) may also result in local metabolic production of formaldehyde. Given the similarities in metabolic processing of HMPA and TMPA, nasal lesions are expected to be a toxic effect of oral TMPA exposure, although direct evidence of such effect is currently lacking. In total, HMPA is judged to be an appropriate analogue for TMPA for deriving screening p-RfDs.

Inhalation Noncancer

No p-RfCs can be derived for TMPA, because p-RfCs were not derived in the PPRTV assessment for HMPA (the selected analogue) because no subchronic inhalation studies were available and the data from the lone chronic inhalation study were not sufficient to support a quantitative dose-response assessment. This lone chronic inhalation study presented substantial uncertainties regarding the experimental design, establishing clear dose-response data (which requires a number of assumptions to be made), and data reporting (U.S. EPA, 2012b).

ORAL NONCANCER TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, HMPA was selected as the analogue for TMPA for deriving a screening subchronic p-RfD. A PPRTV for HMPA is available. The study used to derive the PPRTV subchronic p-RfD for HMPA was a 90-day drinking water study in rats [Keller et al. (1997) as cited in U.S. EPA (2012b)]. The PPRTV for HMPA describes this study as follows:

> *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 1,000 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 consumptions, and mean group water consumption were determined weekly. After 45 and 90 days of treatment, blood samples were collected for 10 rats/sex/group for hematology (erythrocyte, leukocyte, differential leukocyte, platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean hemoglobin concentration) and clinical chemistry (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities, and concentrations of blood urea nitrogen, total protein, 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 weigh tor 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 1,000 ppm on Days 15-92 (see Appendix A, Table A.1 [in U.S. EPA, 2012d]*). 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 1,000-ppm HMPA (see Appendix A, Table A.2* [in U.S. EPA, 2012d] *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 histopathological observations, and the study authors considered these findings to be spurious.*

Comprehensive histopathological 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 1,000 ppm. The study authors rated the severity of the respiratory tract lesions as

normal, minimal, mild, marked, or severe. Table A.3 [in U.S. EPA, 2012d] *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 1,000 ppm. Bilateral testicular atrophy occurred at 1,000 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 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 (1,000 ppm, or 123 mg/kg-day in males).*

The critical effect for the 90-day rat study of HMPA was increased severity of nasal lesions at 100 ppm (15 mg/kg-day in males and 20 mg/kg-day in females). The male NOAEL of 1.2 mg/kg-day was used as the POD in deriving p-RfDs for HMPA (U.S. EPA, 2012b) and is adopted herein as the analogue POD for TMPA.

In the current assessment, the NOAEL of 1.2 mg/kg-day was converted to a human equivalent dose (HED) according to current U.S. EPA (2011c) guidance. In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c), the Agency endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an oral reference dose (RfD). This approach is appropriate for the nasal lesions observed in the study by Keller et al. (1997) as cited in U.S. EPA (2012b) because they occurred following drinking water exposure and are systemic in origin (attributed to metabolism of HMPA in the liver and nasal tissues after absorption from the gut and distribution throughout the body).

Following U.S. EPA (2011c) guidance, the POD for nasal lesions in rats is converted to an HED through the application of a dosimetric adjustment factor (DAF) derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using an average strain-specific reference BW_a of 0.235 kg for male rats and a reference BW_h of 70 kg for humans, the resulting DAF is 0.24 (U.S. EPA, 2011c). Applying this DAF to the NOAEL of 1.2 mg/kg-day yields a POD (HED) as follows:

$$\begin{aligned} \text{POD (HED)} &= \text{NOAEL (mg/kg-day)} \times \text{DAF} \\ &= 1.2 \text{ mg/kg-day} \times 0.24 \\ &= 0.29 \text{ mg/kg-day} \end{aligned}$$

The subchronic p-RfD for TMPA is derived using an interspecies uncertainty factor (UF_A) of 3 because cross-species dosimetric adjustment to a POD (HED) was performed, as described above. Additionally, for the derivation of the screening subchronic p-RfD for TMPA, a database uncertainty factor (UF_D) of 10 was applied to account for the absence of toxicity information for TMPA, and an intraspecies uncertainty factor (UF_H) of 10 was applied to account for human-to-human variability in the absence of information to assess the toxicokinetics and toxicodynamics of TMPA in humans. Thus, the screening subchronic p-RfD for TMPA is derived using the analogue POD (HED) of 0.29 mg/kg-day and a composite uncertainty factor (UF_C) of 300 (reflecting a UF_A of 3, a UF_D of 10, and a UF_H of 10):

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.29 \text{ mg/kg-day} \div 300 \\ &= \mathbf{1 \times 10^{-3} \text{ mg/kg-day}} \end{aligned}$$

Table A-4 summarizes the uncertainty factors for the screening subchronic p-RfD for TMPA.

Table A-4. Uncertainty Factors for the Screening Subchronic p-RfD for TMPA (CASRN 16853-36-4)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following TMPA exposure. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 is applied to account for the absence of reliable toxicity studies evaluating potential systemic, reproductive, and developmental effects of TMPA. The analogue HMPA was used to identify a POD.
UF_H	10	A UF_H of 10 is applied to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of TMPA in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic study was selected as the principal study for the subchronic assessment.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level;
NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose;
TMPA = N,N,N′,N″-tetramethylphosphoramide; UF = uncertainty factor; UF_A = interspecies uncertainty factor;
UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor;
UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

The POD (HED) of 0.29 mg/kg-day identified for nasal lesions in the 90-day drinking water study provided the most sensitive and reliable endpoint in the entire database and was, therefore, selected for the derivation of chronic reference values for HMPA. This analogue POD has been similarly adopted for deriving the screening chronic p-RfD for TMPA. The same uncertainty factors used for the screening subchronic p-RfD (UF_A of 3, UF_D of 10, and UF_H of 10) were applied with an additional subchronic-to-chronic uncertainty factor (UF_S) of 10 to account for extrapolation from a subchronic to a chronic duration. Thus, the screening chronic p-RfD for TMPA was derived using a UF_C of 3,000.

$$\begin{aligned} \textbf{Screening Chronic p-RfD} &= \text{Analogue POD (HED)} \div UF_C \\ &= 0.29 \text{ mg/kg-day} \div 3{,}000 \\ &= \mathbf{1 \times 10^{-4} \text{ mg/kg-day}} \end{aligned}$$

Table A-5 summarizes the uncertainty factors for the screening chronic p-RfD for TMPA.

Table A-5. Uncertainty Factors for the Screening Chronic p-RfD for TMPA (CASRN 16853-36-4)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic or toxicodynamic differences between rats and humans following PMPA exposure. The toxicokinetic uncertainty has been accounted for by calculating an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011c).
UF_D	10	A UF_D of 10 is applied to account for the absence of reliable toxicity studies evaluating potential systemic, reproductive, and developmental effects of TMPA. The analogue HMPA was used to identify a POD.
UF_H	10	A UF_H of 10 is applied to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of TMPA in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a NOAEL.
UF_S	10	A UF_S of 10 is applied because a subchronic study was selected as the principal study for the chronic assessment.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level;
NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose;
TMPA = N,N,N′,N″-tetramethylphosphoramide; UF = uncertainty factor; UF_A = interspecies uncertainty factor;
UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor;
UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

APPENDIX B. BACKGROUND AND METHODOLOGY FOR THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

For reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, there is inadequate information to assess the carcinogenic potential of N,N,N′,N″-tetramethylphosphoramide (TMPA). However, information is available for this chemical which, although insufficient to support a weight-of-evidence (WOE) descriptor and derivation of provisional cancer risk estimates under current guidelines, may be of use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening evaluation of potential carcinogenicity." Appendices receive the same level of internal and external scientific peer review as the provisional cancer assessments in PPRTVs to ensure their appropriateness within the limitations detailed in the document. Users of the information regarding potential carcinogenicity in this appendix should understand that there could be more uncertainty associated with this evaluation than for the cancer WOE descriptors presented in the body of the assessment. Questions or concerns about the appropriate use of the screening evaluation of potential carcinogenicity should be directed to the CPHEA.

The screening evaluation of potential carcinogenicity includes the general steps shown in Figure B-1. The methods for Steps 1−8 apply to any target chemical and they are described in this Appendix. Chemical (TMPA)-specific data for all steps in this process are summarized in Appendix C.

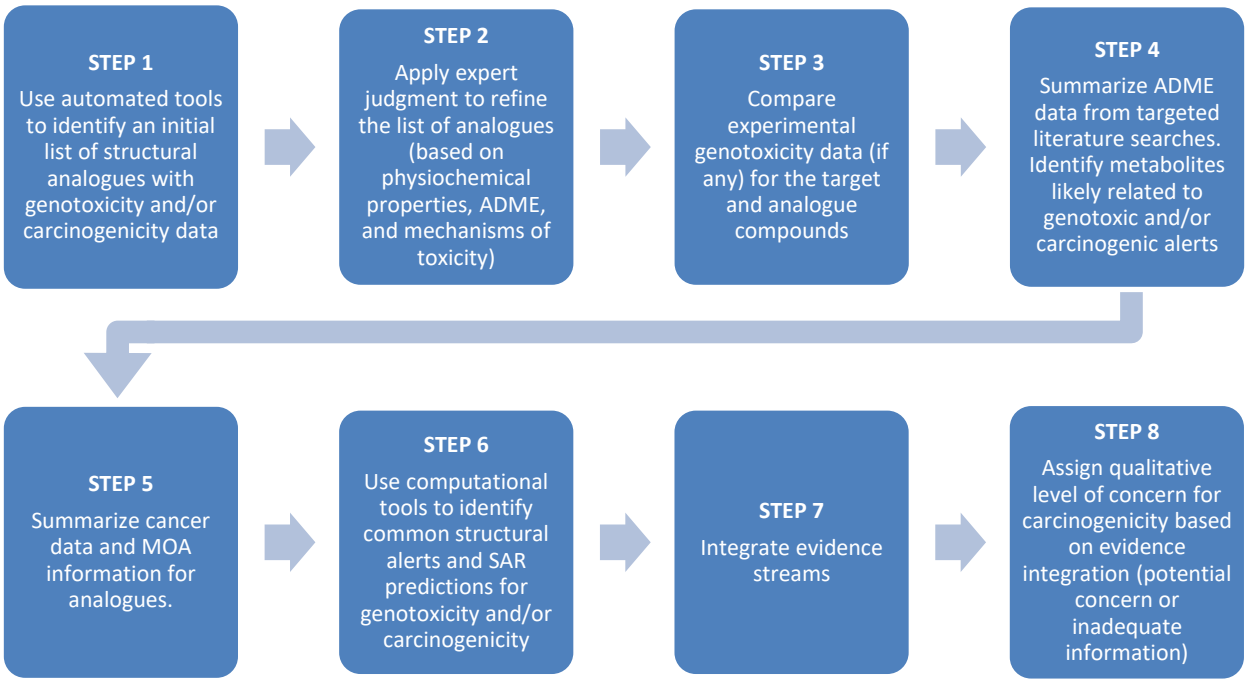


Figure B-1. Steps Used in the Screening Evaluation of Potential Carcinogenicity

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

ChemACE Clustering

The U.S. EPA's Chemical Assessment Clustering Engine [ChemACE; U.S. EPA (2011a)] is an automated tool that groups (or clusters) a user-defined list of chemicals based on chemical structure fragments. The methodology used to develop ChemACE was derived from U.S. EPA's Analog Identification Methodology (AIM) tool, which identifies structural analogues for a chemical based on common structural fragments. ChemACE uses the AIM structural fragment recognition approach for analogue identification and applies advanced queries and user-defined rules to create the chemical clusters. The ChemACE cluster outputs are available in several formats and layouts (i.e., Microsoft Excel, Adobe PDF) to allow rapid evaluation of structures, properties, mechanisms, and other parameters which are customizable based on an individual user's needs. ChemACE clustering has been successfully used with chemical inventories for identifying trends within a series of structurally similar chemicals, demonstrating structural diversity in a chemical inventory, and detecting structural analogues to fill data gaps and/or perform read-across.

For this project, ChemACE is used to identify potential structural analogues of the target compound that have available carcinogenicity assessments and/or carcinogenicity data. An overview of the ChemACE process in shown in Figure B-2.

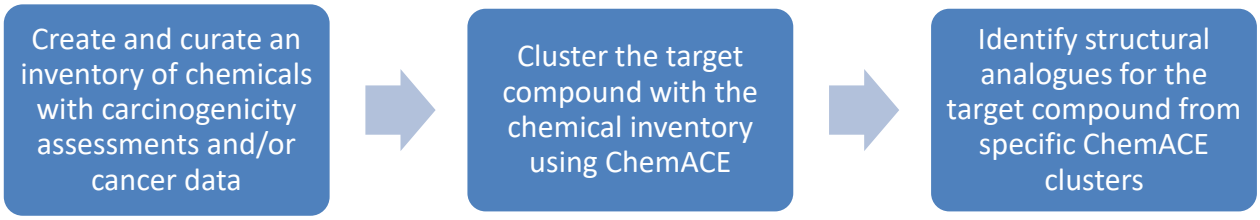


Figure B-2. Overview of ChemACE Process

The chemical inventory was populated with chemicals that have available carcinogenicity assessments and/or carcinogenicity data from the following databases and lists:

- Carcinogenic Potency Database [CPDB; CPDB (2011)]
- Agents classified as Group 1 or 2 carcinogens by the International Agency for Research on Cancer (IARC) monographs (IARC, 2016)
- National Toxicology Program (NTP) Report on Carcinogens [ROC; NTP (2016)]
- NTP technical reports (NTP, 2017)
- Integrated Risk Information System (IRIS) carcinogens (U.S. EPA, 2017)
- California EPA Prop 65 list (CalEPA, 2018a)
- European Chemicals Agency (ECHA) carcinogenicity data available in the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox (OECD, 2018)
- PPRTVs for Superfund (U.S. EPA, 2019)

In total, 2,123 distinct substances were identified from the sources above. For ChemACE clustering, each individual substance needed to meet the following criteria:

1) Substance is not a polymer, metal, inorganic, or complex salt because ChemACE is not designed to accommodate these substances;
2) Substance has a CASRN or unambiguous chemical identification; and
3) Substance has a unique Simplified Molecular Input Line Entry System (SMILES) notation (encoded molecular structure format used in ChemACE) that can be identified from one of these sources:
    a. SRC and DSSTox lists of known SMILES associated with unique CASRNs (the combined lists contained >200,000 SMILES) or
    b. ChemIDplus, U.S. EPA CompTox Chemicals Dashboard, or internet searches.

Of the initial list of 2,123 substances, 201 were removed because they did not meet one of the first two criteria, and 155 were removed because they did not meet the third. The final inventory of substances contained 1,767 unique compounds.

Two separate ChemACE approaches were compared for clustering of the chemical inventory. The restrictive clustering approach, in which all compounds in a cluster contain the same fragments and no different fragments, resulted in 208 clusters. The less restrictive approach included the following rules for remapping the chemical inventory:

- treat adjacent halogens as equivalent, allowing fluorine (F) to be substituted for chlorine (Cl), Cl for bromine (Br), Br for iodine (I);
- allow methyl, methylene, and methane to be equivalent;
- allow primary, secondary, and tertiary amines to be equivalent; and
- exclude aromatic thiols (removes thiols from consideration).

Clustering using the less restrictive approach (Pass 2) resulted in 284 clusters. ChemACE results for clustering of the target chemical (TMPA) within the clusters of the chemical inventory are described in Appendix C.

Analogue Searches in the OECD QSAR Toolbox (DICE Method)

The OECD QSAR Toolbox (Version 4.1) is used to search for additional structural analogues of the target compound. There are several structural similarity score equations available in the Toolbox (DICE, Tanimoto, Kulczynski-2, Ochiai/Cosine and Yule). DICE is considered the default equation. The specific options that are selected for performing this search include a comparison of molecular features (atom-centered fragments) and atom characteristics (atom type, count hydrogens attached and hybridization). Chemicals identified in these similarity searches are selected if their similarity scores exceed 50%.

The OECD QSAR Toolbox Profiler is used to identify those structural analogues from the DICE search that have carcinogenicity and/or genotoxicity data. Nine databases in the OECD QSAR Toolbox (Version 4.1) provide data for carcinogenicity or genotoxicity (see Table B-1).

Analogue search results for the target chemical are described in Appendix C.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
CPDB	The CPDB provides access to bioassay literature with qualitative and quantitative analysis of published experiments from the general literature (through 2001) and from the NTP (through 2004). Reported results include bioassays in rats, mice, hamsters, dogs, and nonhuman primates. A calculated carcinogenic potency (TD_{50}) is provided to standardize quantitative measures for comparison across chemicals. The CPDB contains 1,531 chemicals and 3,501 data points.
ISSCAN	The ISSCAN database provides information on carcinogenicity bioassays in rats and mice reported in sources including NTP, CPDB, CCRIS, and IARC. This database reports a carcinogenicity TD_{50}. The ISSCAN database includes 1,149 chemicals and 4,518 data points.
ECHA CHEM	The ECHA CHEM database provides information on chemicals manufactured or imported in Europe from registration dossiers submitted by companies to ECHA to comply with the REACH regulation framework. The ECHA database includes 9,229 chemicals with almost 430,000 data points for a variety of endpoints including carcinogenicity and genotoxicity. ECHA does not verify the information provided by the submitters.
ECVAM Genotoxicity and Carcinogenicity	The ECVAM Genotoxicity and Carcinogenicity database provides genotoxicity and carcinogenicity data for Ames positive chemicals in a harmonized format. ECVAM contains in vitro and in vivo bacteria mutagenicity, carcinogenicity, CA, CA/aneuploidy, DNA damage, DNA damage and repair, mammalian culture cell mutagenicity, and rodent gene mutation data for 744 chemicals and 9,186 data points.
Cell Transformation Assay ISSCTA	ISSCTA provides results of four types of in vitro cell transformation assays including Syrian hamster embryo cells, mouse BALB/c 3T3, mouse C3H/10T1/2, and mouse Bhas 42 assays that inform nongenotoxic carcinogenicity. ISSCTA consists of 352 chemicals and 760 data points.
Bacterial mutagenicity ISSSTY	The ISSSTY database provides data on in vitro *Salmonella typhimurium* Ames test mutagenicity (positive and negative) taken from the CCRIS database in TOXNET. The ISSSTY database provides data for 7,367 chemicals and 41,634 data points.
Genotoxicity OASIS	The Genotoxicity OASIS database provides experimental results for mutagenicity from "Ames tests (with and without metabolic activation), in vitro CAs and MN, and MLA evaluated in vivo and in vitro, respectively." The Genotoxicity OASIS database consists of 7,920 chemicals with 29,940 data points from 7 sources.
Micronucleus OASIS	The Micronucleus OASIS database provides experimental results for in vivo bone marrow and peripheral blood MNT CA studies in blood erythrocytes, bone marrow cells, and polychromatic erythrocytes of humans, mice, rabbits, and rats for 557 chemicals.

Table B-1. Databases Providing Carcinogenicity and Genotoxicity Data in the OECD QSAR Toolbox (Version 4.1)

Database Name	Toolbox Database Description[a]
Micronucleus ISSMIC	The ISSMIC database provides data on the results of in vivo MN mutagenicity assay to detect CAs in bone marrow cells, peripheral blood cells, and splenocytes in mice and rats. Sources include TOXNET, NTP, and the Leadscope FDA CRADA Toxicity Database. The ISSMIC database includes data for 563 chemicals and 1,022 data points.

[a]Descriptions were obtained from the OECD QSAR Toolbox documentation (Version 4.1) (OECD, 2018).

CA = chromosomal aberration; CCRIS = Chemical Carcinogenesis Research Information System; CPDB = Carcinogenic Potency Database; CRADA = Cooperative Research and Development Agreement; DNA = deoxyribonucleic acid; ECHA = European Chemicals Agency; ECVAM = European Centre for the Validation of Alternative Methods; FDA = Food and Drug Administration; IARC = International Agency for Research on Cancer; ISSCAN = Istituto Superiore di Sanità Chemical Carcinogen Database; ISSCTA = Istituto Superiore di Sanità Cell Transformation Assay Database; ISSMIC = Istituto Superiore di Sanità Micronucleus Database; ISSSTY = Istituto Superiore di Sanità *Salmonella Typhimurium* Database; MLA = mouse lymphoma gene mutation assay; MN = micronuclei; MNT = micronucleus test; NTP = National Toxicology Program; OECD = Organisation for Economic Co-operation and Development; QSAR = Quantitative Structure-Activity Relationship; REACH = Registration, Evaluation, Authorisation and Restriction of Chemicals; TD_{50} = median toxic dose.

STEPS 2−5. ANALOGUE REFINEMENT AND SUMMARY OF EXPERIMENTAL DATA FOR GENOTOXICITY, TOXICOKINETICS, CARCINOGENICITY, AND MODE OF ACTION

The outcome of the Step 1 analogue identification process using ChemACE and the OECD QSAR Toolbox is an initial list of structural analogues with genotoxicity and/or carcinogenicity data. Expert judgment is applied in Step 2 to refine the list of analogues based on physiochemical properties, absorption, distribution, metabolism, and excretion (ADME), and mechanisms of toxicity. The analogue refinement process is chemical-specific and is described in detail for TMPA in Appendix C. Steps 3, 4, and 5 (summary of experimental data for genotoxicity, toxicokinetics, carcinogenicity, and mode of action [MOA]) are also chemical specific (see Appendix C for further details).

STEP 6. STRUCTURAL ALERTS AND STRUCTURE-ACTIVITY RELATIONSHIP PREDICTIONS

Structural alerts and predictions for genotoxicity and carcinogenicity are identified using six freely available structure-based tools (described in Table B-2).

Table B-2. Tools Used to Identify Structural Alerts and Predict Carcinogenicity and Genotoxicity	
Name	**Description[a]**
OECD QSAR Toolbox (Version 4.1)	Seven OECD QSAR Toolbox profiling methods were used, including: • Carcinogenicity (genotox and nongenotox) alerts by ISS (Version 2.3); updated version of the module originally implemented in Toxtree. It is a decision tree for estimating carcinogenicity, based on 55 structural alerts (35 from the Toxtree module and 20 newly derived). • DNA alerts for AMES by OASIS (Version 1.4); based on the Ames mutagenicity TIMES model, uses 85 structural alerts responsible for interaction of chemicals with DNA. • DNA alerts for CA and MNT by OASIS (Version 1.1); based on the DNA reactivity of the CAs TIMES model, uses 85 structural alerts for interaction of chemicals with DNA. • In vitro mutagenicity (Ames test) alerts by ISS (Version 2.3); based on the Mutagenicity module in Toxtree. It is a decision tree for estimating in vitro (Ames test) mutagenicity, based on a list of 43 structural alerts relevant to the investigation of chemical genotoxicity via DNA adduct formation. • In vivo mutagenicity (MN) alerts by ISS (Version 2.3); based on the ToxMic rulebase in Toxtree. The rulebase has 35 structural alerts for in vivo MN assay in rodents. • OncoLogic™ Primary Classification (Version 4.0); "developed by LMC and OECD to mimic the structural criteria of chemical classes of potential carcinogens covered by the U.S. EPA's OncoLogic Cancer Expert System for Predicting the Carcinogenicity Potential" for categorization purposes only, not for predicting carcinogenicity. It is applicable to organic chemicals with at least one of the 48 alerts specified. • Protein binding alerts for chromosomal aberrations by OASIS (Version 1.3); based on 33 structural alerts for interactions with specific proteins including topoisomerases, cellular protein adducts, etc.
OncoLogic (Version 7)	OncoLogic is a tool for predicting the potential carcinogenicity of chemicals based on the application of rules for SAR analysis, developed by experts. Results may range from "Low" to "High" concern level.

ToxAlerts	ToxAlerts is a platform for screening chemical compounds against structural alerts, developed as an extension to the Online Chemical Monitoring Environment (OCHEM: https://ochem.eu) system. Only "approved alerts" were selected, which corresponds to a moderator approving the submitted data. A list of the ToxAlerts found for the chemicals screened in the preliminary batch is below:
	• Genotoxic carcinogenicity, mutagenicity
	  ○ Aliphatic halide (general)
	  ○ Aliphatic halide (specific)
	  ○ Aliphatic halogens
	  ○ Aromatic amine (general)
	  ○ Aromatic amine (specific)
	  ○ Aromatic amines
	  ○ Aromatic and aliphatic substituted primary alkyl halides
	  ○ Aromatic nitro (general)
	  ○ Aromatic nitro (specific)
	  ○ Aromatic nitro groups
	  ○ Nitroarenes
	  ○ Nitro-aromatic
	  ○ Primary and secondary aromatic amines
	  ○ Primary aromatic amine, hydroxyl amine, and its derived esters or amine generating group
	• Nongenotoxic carcinogenicity
	  ○ Aliphatic halogens

Table B-2. Tools Used to Identify Structural Alerts and Predict Carcinogenicity and Genotoxicity	
Name	**Description[a]**
ToxRead (Version 0.9)	ToxRead is a tool designed to assist in making read-across evaluations reproducible. Structural alerts for mutagenicity are extracted from similar molecules with available experimental data in its database. Five similar compounds were selected for this project. The rule sets included: • Benigni/Bossa as available in Toxtree (Version 1) • SARpy rules extracted by Politecnico di Milano, with the automatic tool SARpy • IRFMN rules extracted by human experts at Istituto di Ricerche Farmacologiche Mario Negri • CRS4 rules extracted by CRS4 Institute with automatic tools
Toxtree (Version 2.6.13)	Toxtree estimates toxic hazard by applying a decision tree approach. Chemicals were queried in Toxtree using the Benigni/Bossa rulebase for mutagenicity and carcinogenicity. If a potential carcinogenic alert based on any QSAR model or if any structural alert for genotoxic and nongenotoxic carcinogenicity was reported, then the prediction was recorded as a positive carcinogenicity prediction for the test chemical. The output definitions from the tool manual are listed below: • SA for genotoxic carcinogenicity (recognizes the presence of one of more SAs and specifies a genotoxic mechanism) • SA for nongenotoxic carcinogenicity (recognizes the presence of one or more SAs, and specifies a nongenotoxic mechanism) • Potential *Salmonella typhimurium* TA100 mutagen based on QSAR • Unlikely to be a *S. typhimurium* TA100 mutagen based on QSAR • Potential carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Unlikely to be a carcinogen based on QSAR (assigned according to the output of QSAR8 aromatic amines) • Negative for genotoxic carcinogenicity (no alert for genotoxic carcinogenicity) • Negative for nongenotoxic carcinogenicity (no alert for nongenotoxic carcinogenicity)

Table B-2. Tools Used to Identify Structural Alerts and Predict Carcinogenicity and Genotoxicity

Name	Description[a]
VEGA	VEGA applies several QSARs to a given chemical, as described below: • Mutagenicity (Ames test) CONSENSUS model: a consensus assessment is performed based on predictions of the VEGA mutagenicity models (CAESAR, SARpy, ISS, and *k*-NN) • Mutagenicity (Ames test) model (CAESAR): integrates 2 models: 1 is a trained SVM classifier, and the other is for FNs removal based on structural alerts matching • Mutagenicity (Ames test) model (SARpy/IRFMN): rule-based approach with 112 rules for mutagenicity and 93 for nonmutagenicity, extracted with SARpy software from the original training set from the CAESAR model; includes rules for both mutagenicity and non-mutagenicity • Mutagenicity (Ames test) model (ISS): rule-based approach based on the work of Benigni and Bossa (ISS) as implemented in the software Toxtree (Version 2.6) • Mutagenicity (Ames test) model (*k*-NN/read-across): performs a read-across and provides a qualitative prediction of mutagenicity on *S. typhimurium* (Ames test) • Carcinogenicity model (CAESAR): Counter Propagation Artificial neural network developed using data for carcinogenicity in rats extracted from the CPDB database • Carcinogenicity model (ISS): built implementing the same alerts Benigni and Bossa (ISS) implemented in the software Toxtree (Version 2.6) • Carcinogenicity model (IRFMN/ANTARES): a set of rules (127 structural alerts), extracted with the SARpy software from a data set of 1,543 chemicals obtained from the carcinogenicity database of EU-funded project ANTARES • Carcinogenicity model (IRFMN/ISSCAN-CGX): based on a set of rules (43 structural alerts) extracted with the SARpy software from a data set of 986 compounds; the data set of carcinogenicity of different species was provided by Kirkland et al. (2005)

[a]There is some overlap between the tools. For example, OncoLogic classification is provided by the QSAR Toolbox but the prediction is available only through OncoLogic, and alerts or decision trees were used or adapted in several models (e.g., Benigni and Bossa alerts and Toxtree decision tree) (OECD, 2018).

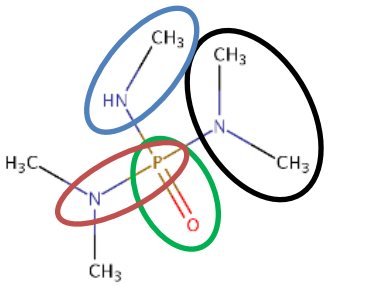
ANTARES = Alternative Non-testing Methods Assessed for REACH Substances; CA = chromosomal aberration; CAESAR = Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations; CPDB = Carcinogenic Potency Database; DNA = deoxyribonucleic acid; EU = European Union; FN = false negative; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; MN = micronucleus; MNT = micronucleus test; OCHEM = Online Chemical Monitoring Environment; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SA = structural alert; SAR = structure-activity relationship; SVM = support vector machine; TIMES = The Integrated MARKEL-EFOM System.

The tool results for the target (TMPA) and analogue compounds are provided in Appendix C.

APPENDIX C. RESULTS OF THE SCREENING EVALUATION OF POTENTIAL CARCINOGENICITY

STEP 1. USE OF AUTOMATED TOOLS TO IDENTIFY STRUCTURAL ANALOGUES WITH CARCINOGENICITY AND/OR GENOTOXICITY DATA

ChemACE clustering was performed as described in Appendix B. The cluster containing N,N,N′,N″-tetramethylphosphoramide (TMPA; less restrictive approach; Cluster 16) also contained pentamethylphosphoramide (PMPA), hexamethylphosphoramide (HMPA), and no other compounds. All of the cluster members have chemical structures that contain: (1) a phosphorus-oxygen double bond, (2) the phosphorus also has three single bonds to nitrogen, and (3) the nitrogen is a secondary or tertiary amide (see Figure C-1).



Secondary amide
Tertiary amide
Nitrogen-phosphorus bond
Phosphorus-oxygen double bond

Figure C-1. Illustration of Common Fragments in Cluster 16 for TMPA

The Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-Activity Relationship (QSAR) Toolbox Profiler was used to identify structural analogues from the DICE analogue search that have carcinogenicity and/or genotoxicity data (see Step 1 methods in Appendix B). The OECD QSAR Toolbox Profiler did not identify any structural analogues of TMPA having carcinogenicity or genotoxicity data (including HMPA). TMPA and HMPA have a similarity score of only 40% because HMPA has three $-N(CH_3)_2$ fragments and TMPA only has only one (see Figure C-2).

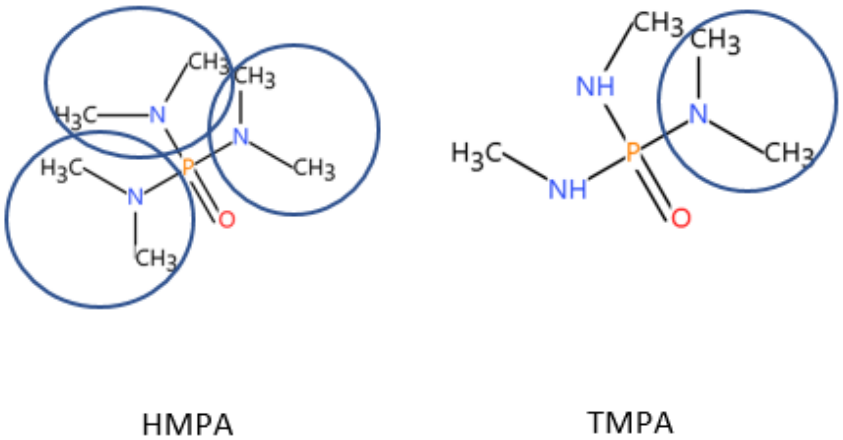


Figure C-2. Illustration of Common Atom-Centered Fragments in the DICE Search

STEP 2. ANALOGUE REFINEMENT USING EXPERT JUDGMENT

Expert chemistry judgment (informed by toxicological expertise) was applied to evaluate whether HMPA is a viable structural analogue for TMPA. TMPA is an *N*-methylated phosphoric acid triamide with four methyl groups located at the N, N, N′, and N″ positions. Experimental toxicokinetic data for HMPA confirm that its predominant metabolic pathway is oxidative demethylation, which results in sequential removal of one methyl group at each of the amide positions. Thus, HMPA is converted to PMPA, then proceeds to TMPA, and finally to N,N′,N″-trimethylphosphoramide (triMPA) (see "Toxicokinetics" section and Appendix A for further discussion). Each demethylation step produces formaldehyde, a known human carcinogen (NTP, 2016). Demethylation by cytochrome P450 (CYP450) has been demonstrated in liver, lung, and nasal cavity tissues, suggesting that carcinogenicity may occur following both inhalation and oral exposure (see Appendix A). HMPA is an appropriate analogue for assessing the carcinogenicity of TMPA because it is a metabolic precursor to the target chemical, and because both compounds are expected to generate the same carcinogenic metabolite, formaldehyde.

STEP 3. COMPARISON OF THE EXPERIMENTAL GENOTOXICITY DATA FOR TMPA AND HMPA

TMPA (unknown isomer) did not induce mitotic recombination in the *Drosophila melanogaster* white/white$^+$ (w/w$^+$) eye mosaic system [Vogel and Nivard (1993); see "Other Data" section in the main body of this PPRTV assessment]. No additional genotoxicity data were available for TMPA.

U.S. EPA (2012b) reported that HMPA was generally negative in bacterial mutagenicity studies using *Salmonella typhimurium* and *Escherichia coli* (with and without metabolic activation), but did produce somatic mutation, mitotic recombination, and sex-linked recessive lethal mutations in *D. melanogaster* and mitotic gene conversion in *Saccharomyces cerevisiae* (with metabolic activation). The effects in *Drosophila* were suggested to fit a pattern seen with crosslinking agents (Bogdanffy et al., 1997). HMPA produced deoxyribonucleic acid (DNA)-protein crosslinks in rat nasal epithelial cells following inhalation exposure (Kuykendall et al., 1995).

HMPA was mutagenic in mouse lymphoma P388F and L5178Y cells (with metabolic activation), but was not mutagenic in Chinese hamster ovary (CHO) or V79 cells (with or without metabolic activation) (U.S. EPA, 2012b). HMPA did not induce chromosome aberrations (CAs) in human lymphocytes or rat liver RL_1 cells (not tested with metabolic activation). Both negative and positive findings (with metabolic activation only) were reported in sister chromatid exchange (SCE) assays in CHO cells. Micronucleus (MN) frequency was increased in human HepG2 cells, but not in human lymphocytes tested without metabolic activation (IARC, 1999).

Genotoxicity studies of HMPA in vivo showed positive findings of CAs in rat peripheral lymphocytes (but not mouse bone marrow), increased MN frequency in rat and mouse bone marrow, and SCE in mouse bone marrow (but not mouse liver) (U.S. EPA, 2012b; IARC, 1999). Mixed findings were reported in the mouse dominant lethal assay (one positive study, one negative study).

STEP 4. TOXICOKINETICS OF TMPA AND HMPA

The toxicokinetics of TMPA and HMPA are described and compared in the "Metabolic Analogues" section of Appendix A. Experimental data show that formaldehyde is released during sequential oxidative demethylation of HMPA to form PMPA, which is further metabolized to TMPA and triMPA.

STEP 5. CARCINOGENICITY OF HMPA AND MODE-OF-ACTION DISCUSSION

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 (U.S. EPA, 2012b). Increased incidences of squamous cell carcinomas of the rat nasal cavity were observed in both sexes (nasal tumors were observed at 400 ppb after 7 months and at 50 ppb after 12 months) (Lee and Trochimowicz, 1982b). The data from this study are not sufficient to support a quantitative cancer dose-response assessment because of uncertainties in the experimental design, in establishing clear dose-response data (which requires a number of assumptions to be made), and in data reporting (U.S. EPA, 2012b).

The HMPA mode of action (MOA) for cancer is not fully established. However, studies indicate a role for metabolism of HMPA likely through CYP450-mediated *N*-demethylation to formaldehyde, which produces tumors in experimental animals following both inhalation and oral exposure (U.S. EPA, 2012b). Whereas, a direct contribution of parent (HMPA) and the subsequently demethylated phosphoramide products (PMPA and TMPA) to HMPA's carcinogenic MOA cannot be ruled out due to a lack of relevant information, the metabolically-induced intracellular release of formaldehyde plays a significant role in HMPA's carcinogenic and mutagenic effects (IARC, 1999; Bogdanffy et al., 1997). This role is supported by studies describing a requirement for metabolism in the production of HMPA-induced DNA-protein crosslinks (Kuykendall et al., 1995). Additionally, data concerning the genotoxicity of PMPA (an expected demethylated methylphosphoramide product) also supports the proposed role for formaldehyde in promoting genotoxicity, because the use of a formaldehyde trapping agent abrogated the mutagenic potential of PMPA after metabolic activation in *S. typhimurium* (Sarrif et al., 1997). The putative MOAs described above would also be relevant for TMPA.

STEP 6. STRUCTURAL ALERTS AND SAR PREDICTIONS FOR TMPA AND HMPA

Structural alerts (SAs) and predictions for genotoxicity and carcinogenicity were identified using computational tools as described in Appendix B. The tool results for TMPA and the analogue compound HMPA are shown in Table C-1.

Table C-1. Heat Map Illustrating the Structural Alert and SAR Prediction Results for TMPA (CASRN 16853-36-4) and HMPA (CASRN 680-31-9)[a]

Tool	Model	TMPA	HMPA
VEGA	Mutagenicity (Ames test) CONSENSUS model—assessment		
	Mutagenicity (Ames test) model (CAESAR)—assessment		
	Mutagenicity (Ames test) model (SARpy/IRFMN)—assessment		
	Mutagenicity (Ames test) model (ISS)—assessment		
	Mutagenicity (Ames test) model (*k*-NN/read-across)—assessment		
	Carcinogenicity model (CAESAR)—assessment		
	Carcinogenicity model (ISS)—assessment		
	Carcinogenicity model (IRFMN/ANTARES)—assessment		
	Carcinogenicity model (IRFMN/ISSCAN-CGX)—assessment		
Toxtree	Negative for genotoxic carcinogenicity		
	Negative for nongenotoxic carcinogenicity		
OncoLogic	OncoLogic (prediction of the carcinogenic potential of the chemical)		

	Model results or alerts indicating no concern for carcinogenicity/mutagenicity.
	Model results outside the applicability domain for carcinogenicity/mutagenicity.
	Model results or alerts indicating concern for carcinogenicity/mutagenicity.

[a]All tools and models described in Appendix B were used. Models with results are presented in the heat map (models without results were omitted).

ANTARES = Alternative Nontesting Methods Assessed for REACH Substances; CAESAR = Computer Assisted Evaluation of Industrial Chemical Substances According to Regulations; CONSENSUS = Consensus Assessment based on multiple models (CAESAR, SARpy, ISS, and *k*-NN); DNA = deoxyribonucleic acid; HMPA = hexamethylphosphoramide; IRFMN = Istituto di Ricerche Farmacologiche Mario Negri; ISS = Istituto Superiore di Sanità; ISSCAN-CGX = Istituto Superiore di Sanità Chemical Carcinogen; *k*-NN = *k*-nearest neighbor; SAR = structure-activity relationship; TMPA = N,N,N′,N″-tetramethylphosphoramide; QSAR = quantitative structure-analysis relationship.

The QSAR models in the VEGA tool indicate no concern for mutagenicity of HMPA in the Ames test, which is consistent with experimental data (see Step 3 above). Similarly, VEGA models also indicated no concern for Ames test mutagenicity for TMPA (no experimental data). While the results of QSAR-driven mutagenicity analysis suggest differing mutagenic capacity of HMPA and TMPA, the production of mutagenic metabolic products (e.g., formaldehyde) cannot be ruled out and may still result in mutagenic activity associated with TMPA/HMPA exposure.

Carcinogenicity models were inconsistent for different tools (i.e., positive in VEGA for HMPA and TMPA, negative for genotoxic and nongenotoxic carcinogenicity for both compounds in Toxtree). However, prediction results for HMPA and TMPA were generally consistent when data were available for both compounds. The OncoLogic results for HMPA indicate a structural alert for "Organophosphorous Type Compounds"; however, the designated level of concern from this tool is based on experimental evidence of HMPA carcinogenicity following inhalation exposure. The OncoLogic output for HMPA states: "The final level of carcinogenicity concern for this compound is 'high–moderate,' if the exposure is by inhalation; otherwise the level of concern is 'marginal.'" OncoLogic did not produce a result for TMPA.

STEP 7. EVIDENCE INTEGRATION FOR SCREENING EVALUATION OF TMPA CARCINOGENICITY

Table C-2 presents the data for multiple lines of evidence pertinent to the screening evaluation of the carcinogenic potential of TMPA. HMPA and TMPA are structurally similar compounds (i.e., both are methylated phosphoric acid triamides) and HMPA is a metabolic precursor to TMPA. HMPA induced mitotic recombination in *Drosophila*, and these results fit a pattern seen with crosslinking agents (Bogdanffy et al., 1997). HMPA produced DNA-protein crosslinks in rat nasal epithelial cells following inhalation exposure (Kuykendall et al., 1995), which is consistent with the release of formaldehyde. TMPA metabolism is also expected to generate formaldehyde, which is the proposed toxic moiety for HMPA carcinogenicity (Jones and Jackson, 1968). Data from the parent phosphoramide (PMPA) detail that use of a formaldehyde trapping agent has been demonstrated to inhibit PMPA-driven mutagenicity, and further supports the requirement for metabolic processing in the ability of PMPA to promote genotoxic activity (Sarrif et al., 1997). TMPA (unknown isomer) did not induce mitotic recombination in *Drosophila*; however, TMPA is anticipated to release less formaldehyde than HMPA during metabolism, which may account for the dissimilar results in this assay. No additional genotoxicity data were available for TMPA. Bacterial mutagenicity findings were negative for HMPA. HMPA produced both positive and negative results in the dominant lethal test and assays evaluating mammalian cell mutagenicity, SCE, MN frequency, and CAs (see Step 3 for details). Computational tools indicated no concern for Ames test mutagenicity for TMPA or HMPA. The results from carcinogenicity models were consistent for both HMPA and TMPA, but were inconsistent for different tools (i.e., positive in VEGA for HMPA and TMPA, negative for genotoxic and nongenotoxic carcinogenicity for both compounds in Toxtree). OncoLogic results indicate a structural alert for HMPA based on experimental evidence of HMPA carcinogenicity following inhalation exposure.

Table C-2. Integration of Evidence for TMPA (CASRN 16853-36-4) and HMPA (CASRN 680-31-9)

Evidence Streams	TMPA	HMPA
Analogue selection and evaluation (see Steps 1 and 2)	NA	Appropriate analogue; both compounds are *N*-methylated phosphoric acid triamides; HMPA is metabolic precursor to TMPA
Experimental genotoxicity data (see Step 3)	TMPA (unknown isomer) did not induce mitotic recombination in the *Drosophila*; no additional studies	Somatic mutation, mitotic recombination, and sex-linked recessive lethal mutations in *Drosophila*; mitotic gene conversion in yeast; DNA-protein crosslinks in rat nasal epithelial cells following inhalation; increased in vivo MN frequency; generally negative in bacterial mutagenicity studies; mixed findings (positive and negative) for in vitro mammalian cell mutagenicity, in vitro MN frequency, SCE (in vitro and in vivo), in vivo CAs, and the mouse dominant lethal assay
ADME evaluation (see Step 4)	Formaldehyde released during sequential oxidative demethylation; HMPA and PMPA are metabolic precursors; triMPA is a metabolite	Formaldehyde released during sequential oxidative demethylation; PMPA, TMPA, and triMPA are metabolites
Cancer data and MOA (see Step 5)	ND	Nasal tumors in male and female rats after chronic inhalation exposure; MOA is not fully known, but intracellular release of formaldehyde may be responsible; DNA-protein crosslink formation following HMPA inhalation provides support for this MOA
Common structural alerts and SAR predictions (see Step 6)	No structural alerts; no concern for mutagenicity (Ames test); carcinogenicity models were inconsistent (i.e., positive in VEGA, negative for genotoxic and nongenotoxic carcinogenicity in Toxtree)	Structural alert for organophosphorus type compounds (based on cancer bioassay data); no concern for mutagenicity (Ames test); carcinogenicity models were inconsistent (i.e., positive in VEGA, negative for genotoxic and nongenotoxic carcinogenicity in Toxtree)

ADME = absorption, distribution, metabolism, and excretion; CA = chromosomal aberration; DNA = deoxyribonucleic acid; HMPA = hexamethylphosphoramide; MN = micronuclei; MOA = mode of action; NA = not applicable; ND = no data; PMPA = pentamethylphosphoramide; SAR = structure-activity relationship; SCE = sister chromatid exchange; TMPA = N,N,N′,N″-tetramethylphosphoramide; triMPA = N,N′,N″-trimethylphosphoramide.

STEP 8. QUALITATIVE LEVEL OF CONCERN FOR TMPA POTENTIAL CARCINOGENICITY

Table C-3 identifies the qualitative level of *concern for potential carcinogenicity* of TMPA based on the multiple lines of evidence described above.

Table C-3. Qualitative Level of Concern for Carcinogenicity of TMPA (CASRN 16853-36-4)		
Level of Concern	**Designation**	**Comments**
Concern for potential carcinogenicity	**Selected**	**HMPA is an appropriate analogue for assessing the carcinogenicity of TMPA because it is a metabolic precursor to the target chemical, and because both compounds generate the same carcinogenic metabolite, formaldehyde. There is *"Suggestive Evidence of Carcinogenic Potential"* for HMPA by the inhalation route of exposure based on increased incidences of squamous cell carcinomas of the rat nasal cavity in both sexes. The intracellular release of formaldehyde, which has been suggested to be responsible for HMPA's carcinogenic effects, would be expected to occur for TMPA as well.**
Inadequate information for assigning qualitative level of concern	NS	NA

HMPA = hexamethylphosphoramide; NA = not applicable; NS = not selected;
TMPA = N,N,N′,N″-tetramethylphosphoramide.

APPENDIX D. REFERENCES

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