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Provisional Peer-Reviewed Toxicity Values for

Pentaerythritol Tetranitrate (PETN)
(CASRN 78-11-5)

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

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Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Daniel D. Petersen, MS, PhD, DABT, ATS, ERT
Center for Public Health and Environmental Assessment, Cincinnati, OH

CONTRIBUTOR

Jeffrey Swartout, MS
Center for Public Health and Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212

PRIMARY INTERNAL REVIEWERS

Elizabeth Owens, PhD
Center for Public Health and Environmental Assessment, Cincinnati, OH

Q. Jay Zhao, PhD, MPH, DABT
Center for Public Health and Environmental Assessment, Cincinnati, OH

ADDITIONAL INTERNAL REVIEWERS

John C. Lipscomb, PhD, DABT, Fellow ATS
Center for Environmental Solutions and Emergency Response, Cincinnati, OH

Jeffrey Swartout, MS
Center for Public Health and Environmental Assessment, Cincinnati, OH

PRIMARY EXTERNAL REVIEW

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

PPRTV PROGRAM MANAGEMENT

Teresa L. Shannon
Center for Public Health and Environmental Assessment, Cincinnati, OH

J. Phillip Kaiser, PhD, DABT
Center for Public Health and Environmental Assessment, Cincinnati, OH

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) website at https://ecomments.epa.gov/pprtv.

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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 PENTAERYTHRITOL TETRANITRATE (CASRN 78-11-5)

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 U.S. Environmental Protection Agency (U.S. EPA) 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. 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://ecomments.epa.gov/pprtv.

1. INTRODUCTION

Pentaerythritol tetranitrate (PETN), CASRN 78-11-5, belongs to the class of compounds known as organic nitrates. It is used mainly as a demolition explosive and in the manufacture of detonating fuses and blasting caps (O'Neil, 2013; Lewis, 2007). Commercial PETN is usually mixed with plasticized nitrocellulose or synthetic rubber because PETN in its pure form is too sensitive to friction and impact (NCBI, 2021). PETN is one of the most powerful explosives known, and along with cyclotrimethylenetrinitramine (RDX), it is the main ingredient in Semtex, a plastic explosive. Additionally, PETN is one of a number of organic nitrates (e.g., nitroglycerin [NTG]) used therapeutically as coronary vasodilators in the treatment of cardiovascular conditions (angina pectoris, acute myocardial infarction, congestive heart failure). However, PETN was removed from most markets as a treatment option in the early 1990s, with the notable exception of Eastern Europe, because clear evidence of its efficacy was lacking [for recent reviews, see Daiber and Münzel (2015); Münzel et al. (2013); Münzel and Gori (2013); O'Neil (2013); Rutherford and Struthers (2013); Daiber et al. (2012); Daiber and Münzel (2010); Daiber et al. (2009); Gori and Daiber (2009); Kosmicki (2009); Bode-Böger and Kojda (2005)]. Its use (or consideration of use) in western countries has re-emerged, and clinical studies continue to re-evaluate its efficacy as a treatment for certain cardiovascular disease conditions (Daiber and Münzel, 2015; Rutherford and Struthers, 2013; Kalidindi et al., 2012; Gori and Daiber, 2009; Bode-Böger and Kojda, 2005). PETN is listed on U.S. EPA's Toxic Substances Control Act's (TSCA) public inventory (U.S. EPA, 2020b), and it is registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2018). PETN is subject to the Section 4 Test Rule (U.S. EPA, 2018b) and Section 12(b) Export Notifications (U.S. EPA, 2016) under TSCA. It is also listed on the 2015 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Substance Priority List (ATSDR, 2016).

Commercial PETN is produced by the continuous nitration of pentaerythritol. In this process, pentaerythritol and nitric acid are fed into a reaction where PETN precipitates, and is then isolated by dilution with water and filtration (NCBI, 2021). The empirical formula for PETN is $C_5H_8N_4O_{12}$, and its structure is shown in Figure 1. Table 1 summarizes its physicochemical properties. PETN is a white crystalline solid at room temperature and is extremely explosive, especially when dry, detonating at around 210°C (NOAA, 2016). PETN's low vapor pressure and Henry's law constant indicate that it is not expected to volatilize from either dry or moist surfaces. If PETN did partition to the atmosphere, its vapor pressure indicates that it would exist there in both the vapor and particulate phases. The estimated half-life of vapor-phase PETN in air by reaction with photochemically produced hydroxyl radicals is 6.6 days. The low water solubility and high soil adsorption coefficient for PETN indicate that it will have low mobility in soil and is not expected to leach to groundwater or undergo runoff after a rain event. Based on tests using microbial cultures isolated from river water and sewage sludge, PETN may undergo some degree of biodegradation in the environment (NCBI, 2021).

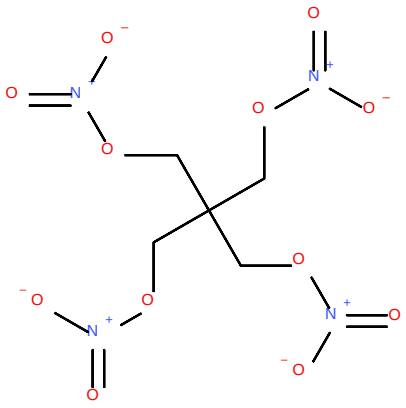

Figure 1. Pentaerythritol Tetranitrate (CASRN 78-11-5) Structure

Table 1. Physicochemical Properties of PETN (CASRN 78-11-5)

Property (unit)	Value[a]
Physical state	Solid
Boiling point (°C)	205–215 (explodes)[b]
Melting point (°C)	141
Density (g/cm^3 at 25°C)	1.73 (predicted average)
Vapor pressure (mm Hg at 25°C)	5.45×10^{-9}
pH (unitless)	NA
pKa (unitless)	NA
Solubility in water (mol/L at 25°C)	1.36×10^{-4}
Octanol-water partition coefficient (log K_{ow})	3.03 (predicted average)
Henry's law constant (atm-m^3/mol at 25°C)	8.43×10^{-7} (predicted average)
Soil adsorption coefficient K_{oc} (L/kg)	120 (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	5.69×10^{-13} (predicted average)
Atmospheric half-life (d)	6.6 (estimated)[c]
Relative vapor density (air = 1)	NA
Molecular weight (g/mol)	316
Flash point (°C)	170 (predicted average)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (PETN, CASRN 78-11-5. https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID2023430. Accessed May 4, 2021). All values are experimental averages unless otherwise noted.
[b]NCBI (2021).
[c]U.S. EPA (2012b).

NA = not applicable; PETN = pentaerythritol tetranitrate.

A previous 2010 PPRTV assessment, which this document supersedes, was available for PETN from the U.S. EPA; no toxicity values are available from other agencies/organizations (see Table 2).

Table 2. Summary of Available Toxicity Values for PETN (CASRN 78-11-5)

Source (parameter)[a, b]	Value	Notes	Reference[c]
Noncancer			
PPRTV (p-RfD)	2×10^{-3} mg/kg-d	Value is for both the subchronic and chronic p-RfDs	U.S. EPA (2010) (archived in 2021)
IRIS	NV	NA	U.S. EPA (2020a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2018a)
ATSDR	NV	NA	ATSDR (2018)
IPCS	NV	NA	IPCS (2020)
CalEPA	NV	NA	CalEPA (2019)
OSHA	NV	NA	OSHA (2020a); OSHA (2020b)
NIOSH	NV	NA	NIOSH (2021)
ACGIH	NV	NA	ACGIH (2020)[d]
Cancer			
PPRTV (p-OSF)	4×10^{-3} $(mg/kg\text{-}d)^{-1}$	Screening p-OSF	U.S. EPA (2010) (archived in 2021)
IRIS	NV	NA	U.S. EPA (2020a)
HEAST	NV	NA	U.S. EPA (2011b)
DWSHA	NV	NA	U.S. EPA (2018a)
NTP	NV	NA	NTP (2016)
IARC	NV	NA	IARC (2018)
CalEPA	NV	NA	CalEPA (2019)
ACGIH	NV	NA	ACGIH (2020)

[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; PPRTV = provisional peer-reviewed toxicity value.
[b]Parameters: p-OSF = provisional oral slope factor; p-RfD = provisional reference dose.
[c]Reference date is the publication date for the database and not the date the source was accessed.
[d]An ACGIH value for pentaerythritol is available; however, the tetranitrate form (PETN) is expected to have sufficiently different solubility (and other ADME parameters) making the applicability of this value unclear.

ADME = absorption, distribution, metabolism, excretion; NA = not applicable; NV = not available; PETN = pentaerythritol tetranitrate.

Literature searches were conducted in January 2016 and updated most recently in April 2021 for studies relevant to the derivation of provisional toxicity values for PETN (CASRN 78-11-5). Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: American Conference of

Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), U.S. EPA TSCATS2/TSCATS8e, U.S. EPA High Production Volume (HPV), Chemicals via IPCS INCHEM, European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Japan Existing Chemical Data Base (JECDB), European Chemicals Agency (ECHA), Organisation for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

2. REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer evidence bases, respectively, for PETN and include all potentially relevant acute and repeated-dose short-term, subchronic, and chronic studies as well as reproductive and developmental toxicity studies. Principal studies used in the PPRTV assessment for derivation of provisional toxicity values are identified in bold. The phrase "statistical significance" and the term "significant," used throughout the document, indicate a *p*-value of < 0.05 unless otherwise specified. Single-dose studies are not time averaged over 24 hours, as the effects of PETN are related more to peak exposure levels than longer-term averages as the half-life is much less than 24 hours. Thus single-dose study dosimetry is expressed as mg/kg rather than mg/kg-day.

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human							
			1. Oral (mg/kg-d)				
Acute	37 healthy adult male volunteers (10 placebo, 9 PETN, 18 other nitrates). Single dose 80-mg tablet.	Once; 0, 1.1 mg/kg	5.7% decrease in systolic blood pressure; no toxicologically relevant side effects reported.	NDr	1.1	Dragoni et al. (2007) (Double-blind clinical trial with random allocation.)	PR
Acute	10 M, 2 F (12 total) heart failure patients: 8 of the subjects received both placebo and PETN treatment, 4 PETN only. Time between treatment administration was determined by "return-to-baseline" hemodynamics. Single dose 40-mg tablet.	Once; 0, 0.57 mg/kg	7−14% decrease in systemic arterial pressure 20−240 min after administration; no toxicologically relevant side effects reported.	NDr	0.57	Amsterdam et al. (1980) (Double-blind clinical trial, crossover design [pre- and post-treatment comparison].)	PR
Acute	14 M, 5 F (19 total) heart failure patients. Single dose 80-mg tablet.	Once; 1.1 mg/kg	8−10% decrease in systemic arterial pressure 1−5 h after administration, compared with pretreatment values; no toxicologically relevant side effects reported.	NDr	1.1	Shah et al. (1980); Shellock et al. (1980) (Clinical trial [pre- and post-treatment comparison].)	PR
Acute	15 M angina patients: all patients received each treatment at random over a 5-d period (1 treatment/d, 2 treatment d, 3 placebo d). Single dose 20-, 40-mg tablets.	Once; 0, 0.29, 0.57 mg/kg	5−10% decrease in systolic blood pressure in supine or standing position 90 min after low- or high-dose administration, compared with the placebo; no toxicologically relevant side effects reported.	NDr	0.29	Dagenais et al. (1969) (Double-blind clinical trial, crossover design.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Acute	10 M angina patients: each subject received both placebo and PETN treatment; 7-d interval between different treatments. Single dose 40-mg tablet.	Once; 0, 0.57 mg/kg	Increased time to exercise-induced angina (implicit vasodilation); no effect on blood pressure; the study authors did not report whether any toxicologically relevant side effects occurred during the trial.	NDr	0.57	Giles et al. (1981) (Double-blind clinical trial [pre- and post-treatment comparison].)	PR
Acute	10 M healthy adult volunteers: each subject received both placebo and PETN treatment; 7-d interval between different treatments. Single dose 2 × 80-mg tablets.	Once; 0, 2.3 mg/kg	Suppression of serum cGMP increase (vasodilation precursor); blood pressure reduced; the study authors did not report whether any toxicologically relevant side effects occurred during the trial.	NDr	2.3	Henstridge et al. (2009) (Double-blind clinical trial [pre- and post-treatment comparison].)	PR
Acute	6 M, 6 F (12 total) healthy adult volunteers: all patients received each treatment at random; 7-d interval between different treatments. Single dose 25-, 50-, or 80-mg tablets.	Once; 0, 0.36, 0.71, 1.1 mg/kg	Implicit vasodilation (reduction in plasma viscosity and capillary erythrocyte velocity); 7% reduction in blood pressure at 1.1 mg/kg-d; the study authors did not report whether any toxicologically relevant side effects occurred during the trial.	0.36	0.71	Bohm and Haustein (1998) (Single-blind clinical trial, crossover design [pre- and post-treatment comparison].)	PR
Short term	5 M healthy and 5 M coronary artery disease patients per group; 3 d. Short-term dose 2 × 150-mg tablets/d.	0, 4.3 mg/kg-d	Suppression of endothelin-1 secretion (suggestive of vasodilation); no effect on blood pressure; the study authors reported no “severe” side effects.	4.3	NDr	Predel et al. (1995) (Double-blind clinical trial.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	42 angina patients (40 M, 2 F). 30-mg extended-release tablets, either 2 or 4/d. 14 d.	0, 0.86, 1.7 mg/kg-d	6/42 had headache or flushing side effects, 2/42 had itching side effect. Reduction in angina attacks, reduction in chest pain, reduction in NTG use. It was not reported which effects occurred at which dose.	NDr	0.86	Roberts (1958) (Double-blind, placebo controlled clinical trial. Both doses grouped together for effects)	PR
Short term	111 pregnant women with abnormal uterine blood flow: 57 placebo, 54 PETN. 12-wk duration, 80 mg 2 times/d.	0, 2.3 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy). No significant increase in toxicologically relevant side effects observed.	NDr	2.3	Schleussner et al. (2014) (Double-blind, placebo-controlled clinical trial with random allocation.)	PR; the 2.3-mg/kg-d dose is considered a NOAEL for side effects.
Short term	71 M, 9 F (80 total) coronary artery disease patients, 40 placebo, 40 PETN (80 mg 3 times/d). 8-wk duration.	0, 3.4 mg/kg-d	Vasodilation measured by brachial arterial blood flow; the study authors did not report whether any toxicologically relevant side effects occurred during the trial.	NDr	3.4	Schnorbus et al. (2010) (Double-blind, placebo controlled clinical trial with random allocation. Side effects recorded in subject diaries, but not reported.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	19 M, 2 F (21 total) subjects completed the study. Each subject (alternating) took PETN, 30-mg tablets 4 times/d or placebo, 4 wk on, 4 wk off. 12-wk duration.	0, 1.7 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); the study authors reported no "detectable differences" in incidence of toxicologically relevant side effects between PETN and placebo treatment. Incidence of side effects similar in both control and treated groups. No significant variations in blood pressure.	NDr	1.7	Aubert et al. (1970) (Double-blind placebo-controlled clinical trial, crossover design.)	PR; the 1.7-mg/kg-d dose is considered a LOAEL for vasodilatory effects and a NOAEL for side effects.
Short term	10 M, 6 F (16 total) coronary artery disease patients and 5 healthy volunteer controls (sex not specified); time-release formulation (Duotrate), either 30-mg tablets 2 times/d or 45-mg tablets 2 times/d. Various durations up to 8 wk.	0, 0.86, 1.3 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); 15/16 vs. 8/12 for the placebo group; side effects were not elevated compared with placebo.	NDr	0.86	Cass and Cohen (1961) (Clinical trial, placebo-controlled, blinding not reported. Extended-release formulation; concurrent NTG treatment.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)							
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Adjusted Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
Short term	**45 M, 27 F (72 total) coronary heart disease patients. Weeks 1−2: placebo Weeks 3−4: 0.86 Weeks 5−6: placebo Weeks 7−8: 0.86, or 1.7 Note: 11 subjects increased to 1.7 mg/kg-d during second 2-wk period due to no improvement in angina symptoms during Weeks 3−4. Dosing 2 × or 4 × 30-mg extended-release tablets every 12 h)**	**0, 0.86, 1.7 mg/kg-d**	**Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); 3/72 patients removed due to side effects at 0.86 mg/kg-d during PETN treatment (2 with headaches, other not specified). No reporting of toxicologically relevant side effects in remaining 69 patients during PETN or placebo treatment. Side effects not considered significant.**	**NDr**	**0.86**	**Hedges and Gordon (1965) (Single-blind placebo-controlled clinical trial, crossover design.)**	**PR, PS; the 0.86-mg/kg-d dose is considered a NOAEL for side effects.**

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	493 M, 162 F, stable angina patients (655 total). 248 M, 79 F placebo (327 total), 245 M, 83 F PETN (328 total). 12-wk duration; 80 mg, 2 times/d.	0, 2.3 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); 10/328 PETN and 7/327 placebo subjects were removed from the study for reasons not well characterized by the study authors. Some toxicologically relevant side effects, similar in number in the PETN group to the placebo group, were reported but otherwise uncharacterized. Thus, the LOAEL applies to the vasodilatation effect, not the side effects.	NDr	2.3	Oelze et al. (2014) (Double-blind, multicenter, clinical trial with placebo control and random allocation. Most subjects were on several other drugs, including beta-blockers, antithrombotics, statins, and ACE inhibitors.)	PR
Short term	50 angina patients, sex not reported; 30 mg 2 or 3 times/d; various durations up to 12 wk.	0, 0.86, 1.3 mg/kg-d; 45 patients received 0.86 mg/kg-d, 5 received 1.3 mg/kg-d. Both time-release (Duotrate) and standard formulation PETN used.	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy). toxicologically relevant side effects: giddiness, light-headedness, vertigo, and palpations reported in 10/50, persistent in 1/50, leading to withdrawal from study.	NDr	0.86	Plotz (1960) (Clinical trial, placebo-controlled, crossover design, blinding not reported. Extended-release formulation of PETN; concurrent NTG treatment. The study authors did not report which side effects occurred at which dose.)	PR; the 0.86-mg/kg dose is considered a NOAEL for side effects.

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	19 M, 1 F, angina patients. Each subject received placebo and both dose levels of PETN. Various durations up to 6 mo. 2 × 10-mg tablets 4 times/d for Dose 1; 3 × 10-mg tablets 4 times/d for Dose 2.	0, 1.1, 1.7 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy). 6/20 patients experienced improved condition at the low dose. Other effects including toxicologically relevant side effects of headaches, drowsiness, and nausea at the high dose (absent when high-dose group reverted to low dose).	1.1 (but see comment about 0.86-mg/kg-d dose)	1.7	Rosenberg and Michelson (1955) (Double-blind, placebo-controlled clinical trial, crossover design; concurrent treatment with NTG; study authors reported no anginal pain reduction at 0.86 mg/kg-d for 5 individuals in a previous trial [i.e., lack of therapeutic effect].)	PR; the 1.1-mg/kg-d dose was considered a NOAEL for side effects and the 1.7-mg/kg-d dose was considered a LOAEL for side effects.
Short term	14 M, 6 F, coronary artery disease patients (12 placebo, 8 PETN) Note: Prior to double-blind study, all subjects had 1 wk titration with PETN to determine personal optimal dose, followed by 1 wk washout with the placebo, then 4 wk with PETN or placebo. PETN doses were 40, 60, or 80 mg, 4 times/d.	0, 2.3, 3.4, 4.6 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); headache, first 2 wk: 6/8 PETN, 3/12 placebo; headache, second 2 wk: 5/8 PETN, 2/12 placebo.	NDr	2.3	Shrivastava et al. (1983) (Double-blind placebo-controlled clinical trial. Actual doses for each subject not reported.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	22 M, 14 F, angina patients. 4-wk duration; 10-mg tablet, 8 times/d.	1.1 mg/kg-d	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); toxicologically relevant side effects were: 5 headache, 5 nausea, 3 dizziness, 1 insomnia, 1 drowsiness. 2 withdrew due to side effects (1 dizziness and palpitation, 1 anorexia and constipation).	NDr	1.1	Edson et al. (1961) (Double-blind clinical trial, no placebo control group.)	PR; the LOAEL applies to both the vasodilatation effect and side effects.
Short term	37 healthy and 27 cardiovascular disease patients, sex not reported. Various durations up to 30 wk, 30−160 mg/d. Doses were increased incrementally for some subjects; others received high dose throughout.	0.43, 0.86, 1.1, 1.4, 1.7, 2.0, 2.3 mg/kg-d; doses linked to effects primarily as averages for specific treatment groups within a range: average (range) = 100 (30−140), 101 (30−160), 112 (30−140), 125 (100−160) mg/d, corresponding to 1.4, 1.6, 1.8 mg/kg-d.	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); 7/10 patients showed partial relief of symptoms at the 100−101-mg/d dose, indicating therapeutic efficacy; decreased systolic blood pressure in 7/21 hypertensive subjects (dose not specified); 1/64 subjects in the 2.0-mg/kg dose group complained of worsening headaches and did not complete the study. No control group data reported.	NDr	NDr	Perlman (1952) (Clinical trial, no placebo control group. Poor reporting of cardiovascular outcomes; cannot determine exact doses or any endpoint.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term to subchronic	21 M, 8 F, arteriosclerosis patients. 7−14 mo durations. 28 subjects, 10-mg tablets, 4/d; 1 subject 10 mg, 3 times/d; 8 subjects increased to 20 mg, 4 times/d.	0.42−0.57, 1.1 mg/kg-d in 8 subjects; no control group.	Vasodilation with potential for reduced blood pressure (implicit from therapeutic efficacy); therapeutic effects for 23/29 patients at 0.57 mg/kg-d were categorized as fair (2), good (18), or excellent (3), based on decreased number of angina attacks and decreased need for NTG; 1/29 had slight nausea at 0.57 mg/kg-d, 1/29 had nausea and headache at 0.57 mg/kg-d.	NDr	0.57	Phillips (1953) (Clinical case series. No control [placebo] group; concurrent treatment with NTG.)	PR; the 0.57-mg/kg-d dose is considered a LOAEL for vasodilatory effects and a NOAEL for side effects.
Short term	6 M, 4 F, inpatients with diseases other than coronary artery disease or other heart conditions. 4-wk duration; 10-mg tablet, 4 times/d, Weeks 1−2; 20 mg, 3 times/d, Weeks 3−4.	2 wk at 0.57 mg/kg-d, 2 wk at 0.86 mg/kg-d	Decreased response to NTG challenge, suggestive of development of cross-nitrate tolerance, toxicological significance uncertain but doubtful. Headaches reported in some individuals but always associated with NTG challenge.	NDr	NDr	Schelling and Lasagna (1967) (Clinical trial [pre- and post-treatment]. No endpoints of toxicological significance for PETN treatment; cannot establish LOAEL or NOAEL because of comorbidities and lack of placebo group.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Short term	10 healthy volunteers per group (60 mg, 3 times/d), 7 d.	0, 2.6 mg/kg-d	6% decrease in systolic blood pressure and 18% increase in heart rate following PETN administration, compared with pretreatment. No toxicologically relevant side effects reported.	NDr	2.6	Jurt et al. (2001) (Double-blind clinical trial with random allocation [pre- and post-treatment comparison with NTG].)	PR; the 2.6-mg/kg-d dose is considered a LOAEL for vasodilatory effects and a NOAEL for side effects.
Short term	28 M healthy volunteers: 80 mg 3 times/d for 6 d	3.4 mg/kg-d (subjects were their own controls)	6% decrease in systolic blood pressure, 7% decrease in diastolic blood pressure, and increased blood flow in forearm following PETN therapy (compared with pretreatment). The study authors did not report whether any toxicologically relevant side effects occurred during the trial.	NDr	3.4	Gori et al. (2003) (Double-blind clinical trial with random allocation [pre- and post-treatment comparison with NTG].)	PR; the 3.4-mg/kg-d dose is considered a LOAEL for vasodilatory effects and a NOAEL for side effects.
Short term	111 pregnant women (54 PETN, 57 placebo) at mid-gestation (average gestational age at start = 21.5 wk). 0 or 80 mg 2 times/d for 88−90 d	0, 2.1 mg/kg-d; assuming an average body weight of 75 kg for pregnant women [see Table 8-29 in U.S. EPA (2011a)]	Vasodilation with potential for reduced blood pressure (implicit from the therapeutic effect of increased uteroplacental and fetoplacental perfusion).	NDr	2.1	Bowkalow et al. (2018) (Double-blind clinical trial with random allocation.)	PR; side effects not reported.
2. Inhalation (mg/m^3)							
ND							

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)							
Category[a]	**Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses**	**Adjusted Dosimetry[b]**	**Critical Effects**	**NOAEL[b]**	**LOAEL[b]**	**Reference (comments)**	**Notes[c]**
Animal							
			1. Oral (mg/kg-d)				
Short term	5 M/5 F, F344/N rat, diet, 7 d/wk, 14 d; 0, 620, 1,240, 2,500, 5,000, or 10,000 ppm.	M: 0, 65.7, 129.0, 347.8, 674, 1,110; F: 0, 79.0, 168.0, 355.7, 635, 1,310	No compound-related toxicologically relevant effects on mortality, clinical signs, body weight, or gross necropsy.	1,310 (F)	NDr	Bucher et al. (1990); NTP (1989)	PR
Short term	5 M/5 F, B6C3F1 mouse, diet, 7 d/wk, 14 d; 0, 620, 1,240, 2,500, 5,000, or 10,000 ppm.	M: 0, 173, 308.7, 539.7, 1,380, 2,600; F: 0, 187, 556.9, 703.1, 1,800, 2,530	58−85% decrease in body-weight gain in females; 13% decrease in terminal body weight at ≥1,800 mg/kg-d.	703.1 (F)	1,800 (F)	Bucher et al. (1990); NTP (1989)	PR
Short term	6 (sex not specified), mongrel dog, gavage, 7 d/wk, 3 wk; 30 mg/d.	1.3	Coronary effects of uncertain biological significance (increased coronary vascular resistance and mechanical efficiency; reduced coronary blood flow, left ventricular oxygen consumption, and left ventricular work).	NDr	NDr	Bender et al. (1963) (Cannot determine toxicological relevance or lack thereof because effects are opposite of expected biological action [vasodilation].)	PR
Subchronic	10 M/10 F, F344/N rat, diet, 7 d/wk, 14 wk; 0, 620, 1,240, 2,500, 5,000, or 10,000 ppm.	M: 0, 39.1, 88.04, 190.0, 330, 630; F: 0, 42.8, 85.56, 200.0, 370, 830	Significant changes in body-weight gain and relative brain weight at concentration ≥200 mg/kg-d.	85.56 (F)	200.0 (F)	Bucher et al. (1990); NTP (1989)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Subchronic	10 M/10 F, B6C3F1 mouse, diet, 7 d/wk, 13 wk; 0, 620, 1,240, 2,500, 5,000, or 10,000 ppm.	M: 0, 109, 302.6, 362.5, 925, 2,140; F: 0, 172, 306.3, 632.5, 1,340, 3,120	No observed noncancer effects. A hepatocellular adenoma was observed in 1 female mouse at 3,120 mg/kg-d.	3,120 (F)	NDr	Bucher et al. (1990); NTP (1989)	PR
Chronic	50 M/50 F, F344/N rat, diet, 7 d/wk, 2 yr; M: 0, 5,000, or 10,000 ppm; F: 0, 1,240, or 2,500 ppm.	M: 0, 240, 490; F: 0, 80, 165	No observed effects.	490 (M)	NDr	Bucher et al. (1990); NTP (1989)	PR
Chronic	50 M/50 F, B6C3F1 mouse, diet, 7 d/wk, 2 yr; 0, 5,000, or 10,000 ppm.	M: 0, 810, 1,620; F: 0, 1,020, 1,936	No observed effects.	1,936 (F)	NDr	Bucher et al. (1990); NTP (1989)	PR
Chronic	45 M + F (sex ratio not reported), albino rat, diet, 7 d/wk, 1 yr; 0 or 2 mg/kg-d.	0, 2	No observed effects.	NDr	NDr	Donahue (1944) (Confidence in study is low due to 50% mortality from parasitic infection in the colony.)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for PETN (CASRN 78-11-5)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Study Duration, Reported Doses	Adjusted Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Reproductive/ Developmental	10 M/10 F, Sprague Dawley rat, gavage in corn oil, 7 d/wk, 28 d in males (premating through mating), up to 56 d in females (premating through PND 3); 0, 100, 500, or 1,000 mg/kg-d.	0, 100, 500, 1,000	No observed effects.	1,000	NDr	Quinn et al. (2009) (Study did not report mating/fertility indices.)	PR
2. Inhalation (mg/m^3)							
ND							

[a]Duration categories are defined as follows: Acute = exposure for ≤24 hours; short term = repeated exposure for >24 hours to ≤30 days; long term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (>30 days up to approximately 90 days in typically used laboratory animal species); and chronic = repeated exposure for >10% lifespan for humans (>~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Dosimetry: Doses are presented as ADDs (mg/kg-day), except single-dose studies where mg/kg doses are presented. Where applicable, reported body-weight data were used if available; if not, reference body-weight values for rodents reported by U.S. EPA (1988) were used; for humans, a default value of 70 kg was used, except for pregnant women, where 75 kg was used.
[c]Notes: PR = peer reviewed; PS = principal study.

ACE = angiotensin-converting enzyme; ADD = adjusted daily dose; cGMP = cyclic guanosine monophosphate; F = female(s); LOAEL = lowest-observed-adverse-effect level; M = male(s); ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level; NTG = nitroglycerin; PETN = pentaerythritol tetranitrate; PND = postnatal day.

Table 3B. Summary of Potentially Relevant Cancer Data for PETN (CASRN 78-11-5)					
Category	**Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**Reference**	**Notes[b]**
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
Carcinogenicity	**50 M/50 F, F344/N rat, diet (0, 5,000, 10,000 ppm M: 0, 1,240, 2,500 ppm F); 2 yr**	**M: 0, 67.2, 137; F: 0, 20, 41.3**	**Increases in Zymbal and thyroid gland tumors (dose related in females)**	**Bucher et al. (1990); NTP (1989)**	**PR, PS**
Carcinogenicity	50 M/50 F, B6C3F1 mouse, diet (0, 5,000, 10,000 ppm); 2 yr	M: 0, 122, 243; F: 0, 153, 290.4	No exposure-related neoplastic lesions	Bucher et al. (1990); NTP (1989)	PR
2. Inhalation (mg/m^3)					
ND					

[a]Dosimetry: The units for oral exposures are expressed as HEDs (mg/kg-day); HED = adjusted daily animal dose (mg/kg-day) × ($BW_a \div BW_h)^{1/4}$ (U.S. EPA, 2005), using study-specific TWAs (for the control, low-, and high-dose groups, respectively) of 0.433, 0.419, and 0.413 kg for male rats; 0.289, 0.276, and 0.284 kg for female rats; 0.0371, 0.0354, and 0.0369 kg for male mice; 0.0363, 0.0332, and 0.0368 kg for female mice; and 70 kg for humans (U.S. EPA, 2011c).
[b]Notes: PR = peer reviewed; PS = principal study.

BW = body weight; F = female(s); HED = human equivalent dose; M = male(s); ND = no data; PETN = pentaerythritol tetranitrate; TWA = time-weighted average.

2.1. HUMAN STUDIES

2.1.1. Oral Exposures

Data regarding oral PETN exposure in humans are available from numerous clinical studies. The therapeutic effects of oral PETN administration are similar to other nitrovasodilators that increase coronary blood supply to the heart and decrease myocardial oxygen demands through preferential dilation of large veins and arteries via smooth muscle relaxation (Klemenska and Beresewicz, 2009; Kosmicki, 2009; Daiber et al., 2008; Murad, 1990). Historically, the typical oral dosage of PETN used to treat angina pectoris has been 10−80 mg as a tablet up to 4 times daily or 30−80 mg as a sustained release capsule every 12 hours [Murad (1990); PDR (1987) as cited in NTP (1989)], with a total daily dose ranging from 30−320 mg/day (0.43−4.6 mg/kg-day for a 70-kg adult). Daiber and Münzel (2015) reported a usage of 50−80 mg per dose (therapeutic action of 8−12 hours), and recent clinical trials have evaluated daily doses of 160−240 mg/day (2.3−3.4 mg/kg-day for a 70-kg adult) (Münzel et al., 2014; Schleussner et al., 2014; Schnorbus et al., 2010). As noted in the "Mode of Action" section below, the therapeutic vasodilatory action of organic nitrates, including PETN, are attributed to their active intermediate, nitric oxide (NO) [reviewed by Daiber and Münzel (2015); Münzel et al. (2013); Kosmicki (2009); Daiber et al. (2008); Bode-Böger and Kojda (2005); Murad (1990)]. As discussed in the "Metabolism" section below, NO is released during PETN metabolism.

Most of the clinical studies in the PETN literature were designed to test the effectiveness of a generally continuous intake of the drug in alleviating angina pectoris. Dosing protocols consisted of administering 3 or 4 immediate-release tablets 3 or 4 times per day or 2 delayed/sustained-release tablets every 12 hours for durations ranging from 3 days to 30 weeks. Given the half-life of PETN for humans in the range of 4−8 hours (Weber et al., 1995) and a therapeutic action duration of 8−12 hours (Daiber and Münzel, 2015), the typical dosing protocols should result in a relatively constant effective internal concentration. Presentation of study results was almost always focused on symptomology rather than on explicit measures of specific endpoints, such as vasodilation or blood pressure. Therefore, the effectiveness of PETN in eliciting the primary therapeutic effect—vasodilation—is largely implicit in these studies for findings of efficacy in alleviating clinical symptoms. The effect (or lack of effect) on blood pressure, the endpoint of greatest concern for environmental exposures to the general population, was infrequently mentioned. Several acute-exposure studies, however, were designed to study vasodilation and blood pressure effects (Henstridge et al., 2009; Dragoni et al., 2007; Bohm and Haustein, 1998; Amsterdam et al., 1980; Shellock et al., 1980; Dagenais et al., 1969). In all those studies, blood pressure reductions were observed; see Table 3A.

The studies using multiple daily treatment protocols ("continuous-exposure" studies) generally reported efficacy of PETN in alleviating angina symptoms at the lowest treatment levels, which are designated as LOAELs in the general population for the critical effect of vasodilation, with the potential for reduced blood pressure. Referring to Table 3A, short-term to subchronic exposure-duration LOAELs for vasodilation in the range of 0.57−3.4 mg/kg-day (assuming a 70 kg adult) have been established (Oelze et al., 2014; Schleussner et al., 2014; Schnorbus et al., 2010; Shrivastava et al., 1983; Aubert et al., 1970; Hedges and Gordon, 1965; Cass and Cohen, 1961; Edson et al., 1961; Plotz, 1960; Rosenberg and Michelson, 1955; Phillips, 1953). In all but one of these studies, vasodilation was implicit (based on therapeutic efficacy). The one exception is the study of Schnorbus et al. (2010), which determined vasodilation by an increase in brachial artery blood flow at a dose level of 3.4 mg/kg-day. Two of the continuous-exposure studies established LOAELs for decreased blood pressure of 2.6 mg/kg-day (Jurt et al., 2001) and 3.4 mg/kg-day (Gori et al., 2003). LOAELs for two other

continuous-exposure studies could not be established because either the treatment levels associated with the reported effects were not specified (Perlman, 1952) or the toxicological relevance of the reported effects could not be determined (Schelling and Lasagna, 1967). In the former case (Perlman, 1952), the therapeutic efficacy was evident (implicit vasodilation) and reduced blood pressure in 7/21 hypertensive subjects was reported, but the exact doses were not specified among several dose levels used, ranging from 30 mg/day to 160 mg/day. In the latter case (Schelling and Lasagna, 1967), the toxicological significance of the reported endpoint (decreased response to NTG challenge) could not be determined (see Table 3A). No NOAELs were identified in any of the continuous-exposure studies, although a NOAEL of 0.86 mg/kg-day was implied by Rosenberg and Michelson (1955), who reported no anginal pain reduction at 0.86 mg/kg-day for five individuals in a previous trial. A confounding factor in several of the studies was concurrent treatment with NTG or other drugs affecting cardiovascular activity (Oelze et al., 2014; Cass and Cohen, 1961; Plotz, 1960; Rosenberg and Michelson, 1955; Phillips, 1953). Thus, determining the exact contribution of PETN to the efficacy of the treatment in these studies is problematic. Notably, the lowest apparent LOAEL of 0.57 mg/kg-day was determined from the Phillips (1953) study, with concurrent NTG treatment, which excludes this value for consideration as a POD.

Side Effects

The term "side effect" is used in the document to largely include toxicologically relevant symptoms expressed by patients, including headache, nausea, dizziness, etc. This is distinct from hemodynamic signs, including blood pressure, heart rate, blood flow, etc., measured in patients. Side effects associated with therapeutic use of organic nitrates (including PETN) are well characterized and virtually all are considered to be secondary to the primary action of vasodilation [reviewed by Daiber et al. (2008); Bode-Böger and Kojda (2005); Murad (1990)]. In double-blind, placebo-controlled, randomized clinical trials following oral PETN administration, study participants exhibited side effects such as headaches (see also Table B-1) at NOAEL doses as low as 1.1 mg/kg-day (Rosenberg and Michelson, 1955). Other evidence from non-placebo-controlled studies, or inadequately reported studies, show increases in side effects at doses >2.3 mg/kg-day (Schelling and Lasagna, 1967). Evidence from the placebo-controlled studies of greater than 4 weeks showed NOAELs for side effects ranging from 0.86−2.3 mg/kg-day (Schleussner et al., 2014; Aubert et al., 1970; Hedges and Gordon, 1965). Additional data from studies lacking a placebo control or inadequately reported studies show putative NOAELs for side effects in the same range (0.57−2.3 mg/kg-day) [e.g., Phillips (1953); Perlman (1952)]. Headaches, sometimes severe, and nausea due to cerebral vasodilation are the most common side effects. Side effects generally occurred at doses higher than those associated with the therapeutic effect of vasodilation, though the NOAELs for side effects overlap the NOAELs and LOAELs for the primary vasodilation effect, and in Edson et al. (1961), the LOAEL for side effects was the same as the LOAEL for vasodilatation.

Blood Pressure Effects

Of particular interest is the potential for a reduction in blood pressure, which, although perhaps a beneficial effect for some cardiac patients, is a detrimental effect in the general population, particularly for those individuals susceptible to hypotension. The latter would include pregnant women, infants, the elderly, those with diabetes, and men on erectile dysfunction medication, among others. Blood pressure, however, is rarely measured in rodent studies. There are other studies in the database supporting this effect including the work of Bender et al. (1963) who reported mean arterial pressure in dogs, and the work with spontaneously hypertensive rats (Commarato et al., 1973) and related studies, see below.

2.1.2. Single-Dose (Acute) Studies

The human clinical studies reporting decreases in blood pressure have largely been single-dose administrations designed to study the acute hemodynamic activity of PETN, rather than as treatment for angina pectoris. The studies have been conducted with cardiac patients (Giles et al., 1981; Amsterdam et al., 1980; Shellock et al., 1980; Dagenais et al., 1969), pregnant women (Henstridge et al., 2009), and healthy volunteers (Henstridge et al., 2009; Dragoni et al., 2007; Bohm and Haustein, 1998) alike. Because of uncertainties of the effective half-life of PETN, the dosimetry here is shown as actual dose. Five of the seven single-dose studies reported reduced systolic (or "systemic") blood pressure in the range of 5−14% at doses ranging from 20−80 mg. In one double-blind study, Dagenais et al. (1969), reported a 5−10% reduction in blood pressure for 15 angina patients following a single dose of 20 mg PETN.[1] Another study showing acute effects of PETN on hemodynamic parameters is Amsterdam et al. (1980), who reported a 7−14% reduction in blood pressure for 12 healthy volunteers given a single dose of 40 mg (also a double-blind design). In addition, Bohm and Haustein (1998) reported a 7% reduction in blood pressure at a dose of 80 mg for 12 healthy volunteers, but no effects at doses of 25 or 50 mg. In a study in pregnant women, significant reductions in blood pressure and compensatory increase in heart rate occurred within 15 minutes of treatment of 160 mg (Henstridge et al., 2009). Conversely, one of the single-dose studies reported no effect on blood pressure at doses of 40 mg in 10 angina patients (Giles et al., 1981). These effects on hemodynamic parameters represent the proximal effects of PETN, and because they appear in acute as well as longer term studies, indicate less of an effect for duration of exposure on the severity of the effect than is typically the case.

2.1.3. Continuous Exposure Studies

Only 3 (Gori et al., 2003; Jurt et al., 2001; Perlman, 1952) of the 16 multiple-daily-dose studies reported reductions in blood pressure. Two of the studies (Gori et al., 2003; Jurt et al., 2001) were designed to evaluate PETN for development of tolerance and potential for oxidative stress, relative to NTG, rather than to evaluate the clinical efficacy of PETN for treatment of angina. The two studies were conducted in the same laboratory with healthy volunteer subjects and used double-blind protocols (Gori et al., 2003; Jurt et al., 2001). Although Jurt et al. (2001) used a separate control group, the subjects in the Gori et al. (2003) study were their own controls (before and after measurements). Gori et al. (2003) found that both PETN and NTG increased forearm blood flow, but that, unlike NTG, repeated PETN treatment was not associated with the development of tolerance or presence of oxidative stress markers. Jurt et al. (2001) reported an average 6% reduction in systolic blood pressure associated with PETN treatment at 2.6 mg/kg-day (60 mg, 3 times daily for 7 days). Gori et al. (2003) confirmed the vasodilatory action and lack of development of tolerance for PETN and reported a 6−7% reduction in both systolic and diastolic blood pressure for 28 healthy male volunteers administered PETN at 3.4 mg/kg-day (80 mg, 3 times daily for 6 days). Both sets of investigators reported no side effects. The third multiple-daily-dose study reporting blood pressure effects was a clinical trial investigating the efficacy of PETN for 27 cardiovascular patients and the potential for side effects in 37 healthy volunteers (Perlman, 1952) at dose levels from 0.43−2.3 mg/kg-day, with treatment durations up to 30 weeks. Perlman (1952) reported that systolic blood pressure was reduced in 7 of 21 hypertensive subjects, presumably from the cardiovascular patient group (but not specified as such); neither the magnitude of the decrease nor the effective dose level(s) was reported. Of the other 13 multiple-daily-dose studies, only 2 specifically reported that blood

[1]Single doses of 20 or 40 mg or placebo (randomized) were administered daily over a 5-day period (two treatment days, three placebo days), but blood pressure measurements were taken immediately after each dose.

pressure was not affected (Schleussner et al., 2014; Predel et al., 1995). There was no mention of blood pressure endpoints in the remaining 11 studies.

2.1.4. Inhalation Exposures

No human studies following inhalation exposure to PETN have been identified.

2.2. ANIMAL STUDIES

2.2.1. Oral Exposures

Short-Term Studies

Bucher et al. (1990); NTP (1989) (Rat Study)

F344/N rats (five/sex/dose) were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm of National Formulary (NF) Grade PETN (PETN NF), a 1:4 formulation of PETN (purity >99%) and D-lactose monohydrate, typically used in human therapeutics, for 14 days. Equivalent PETN concentrations were 0, 620, 1,240, 2,500, 5,000, or 10,000 ppm, respectively. Based on the average of the reported initial and final body weights and food-consumption data from Day 7, the average daily consumption of PETN calculated for this review was 65.7, 129.0, 347.8, 674, or 1,110 mg/kg-day, respectively, in males and 79.0, 168.0, 355.7, 635, or 1,310 mg/kg-day, respectively, in females. The animals were observed twice daily for mortality and clinical signs of toxicity. Body weight was measured weekly and food consumption monitored throughout the study. Gross necropsy was performed on all animals at sacrifice.

Terminal body weights were within 5% of control in all treated groups (see Table B-2). The study authors indicated that there were “no clinical signs or toxic lesions” related to exposure. No further details were provided.

The NOAEL of 1,310 mg/kg-day in females is identified based on a lack of reported effects.

Bucher et al. (1990); NTP (1989) (Mouse Study)

B6C3F1 mice (five/sex/dose) were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm PETN NF (0, 620, 1,240, 2,500, 5,000, or 10,000 ppm PETN) for 14 days. Based on the average of the reported initial and final body weights and food-consumption data from Day 7, the average daily consumption of PETN calculated for this review was 173, 308.7, 539.7, 1,380, or 2,600 mg/kg-day, respectively, in males and 187, 556.9, 703.1, 1,800, or 2,530 mg/kg-day, respectively, in females. The animals were observed twice daily for mortality and clinical signs of toxicity. Body weight was measured weekly and food consumption monitored throughout the study. Gross necropsy was performed on all animals at sacrifice. Microscopic histopathology was limited to the kidney from the control and the two highest dose groups.

All mice survived until sacrifice. No clinical signs of toxicity were reported. Terminal body weight was significantly decreased by 13% in the highest dosed females, compared with control; body-weight gain significantly decreased by 58−85% in females at ≥1,800 mg/kg-day (statistics performed for this review; see Table B-2). The study authors indicated that no exposure-related gross or microscopic lesions were observed.

A NOAEL of 703.1 mg/kg-day and a LOAEL of 1,800 mg/kg-day are identified based on a statistically significant 58% decrease in terminal body-weight gain in female mice.

Bender et al. (1963)

Six mongrel dogs (sex not specified) were administered PETN at a dose of 30 mg via gavage daily for 3 weeks (vehicle and purity not reported). Based on the average reported body weights (15.0−30.5 kg), the estimated daily dose is 1.3 mg/kg-day. Body weight, respiratory rate and ventilator volume, blood oxygen content, and various cardiovascular endpoints (coronary blood flow, cardiac output, oxygen consumption, left ventricular work, mean arterial pressure, mean pulmonary artery pressure, mean coronary sinus pressure, and mechanical efficiency) were determined before the daily PETN administration and after the final PETN administration on Day 21.

A statistically significant 2.3% decrease in body weight was observed following PETN exposure, compared with pre-exposure values. No significant changes were observed in respiratory rate, ventilator volume, or blood oxygen content. The observed decrease in body weight is not considered biologically relevant because the effect was small (<10%). No significant changes were observed in systemic blood pressure values. Significant increases were observed in local cardiac effects including coronary vascular resistance and mechanical efficiency, while significant reductions were observed in other local cardiovascular indicators including coronary blood flow, left ventricular oxygen consumption, and left ventricular work. The coronary fractional flow (coronary blood flow/cardiac output) was significantly decreased by 37%. Because the coronary effects are in the opposite direction of the expected action of PETN (vasodilation), the biological significance of the observed cardiac effects is not clear.

Because of the uncertainty in the biological significance of the cardiac effects, a NOAEL or LOAEL cannot be identified for this study.

Subchronic Studies

Bucher et al. (1990); NTP (1989) (Rat Study)

F344/N rats (10/sex/dose) were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm of PETN NF (0, 620, 1,240, 2,500, 5,000, or 10,000 ppm PETN) for 14 weeks. Based on the reported daily food consumption per kilogram of body weight at Week 7, the average daily consumption of PETN calculated for this review was 39.1, 88.04, 190, 330, or 630 mg/kg-day, respectively, in males and 42.8, 85.56, 200, 370, or 830 mg/kg-day, respectively, in females. The animals were observed twice daily for clinical signs of toxicity. Body weights were recorded at study initiation, once per week during the study, and at necropsy. Feed consumption was measured 2−3 days per week. At necropsy, blood was collected for whole-blood methemoglobin (MetHb) concentration. All animals were grossly examined, and the brain, heart, right kidney, liver, lungs, and thymus were removed and weighed. Comprehensive histological examinations were conducted in the control and highest dose groups of both sexes; evaluations at lower doses were limited to the Zymbal gland in females at 370 mg/kg-day.

No mortalities or clinical signs of toxicity were reported. A statistically significant 6−7% decrease in terminal body weight was observed in females at ≥370 mg/kg-day, and a statistically significant decrease in total body-weight gain was observed in females at ≥200 mg/kg-day (12% at 200 mg/kg-day, 17% at 370 mg/kg-day, and 18% at 830 mg/kg-day), compared with controls (statistics performed for this review; see Table B-3). No body-weight effects were observed in males (see Table B-3). MetHb levels were <1% in all control and exposed groups. In females, relative brain weights were increased significantly by 6−8% at ≥200.0 mg/kg-day, compared with controls, and relative kidney weights were significantly

increased by 6% at 830 mg/kg-day, compared with controls (see Table B-3); absolute organ weights were not reported. No additional organ-weight effects were noted in females, and no dose-related organ-weight effects were observed in males (see Table B-3). No exposure-related non-neoplastic gross or histopathological lesions were observed. An adenoma of the Zymbal gland was observed in one female rat at 830 mg/kg-day.

The decreases in body weight for females did not exceed 10% and are not considered to be biologically significant. The increases in relative kidney weights for females are also not considered toxicologically relevant because they were less than 10%. The increases in relative brain weight and the 12% decrease in body-weight gain are considered to be toxicologically relevant. The NOAEL of 85.56 and LOAEL of 200 mg/kg-day are identified based on increased relative brain weight and decreased body-weight gain in female rats.

Bucher et al. (1990); NTP (1989) (Mouse Study)

B6C3F1 mice (10/sex/dose) were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm PETN NF (0, 620, 1,240, 2,500, 5,000, or 10,000 ppm PETN) for 13 weeks. Based on the average of the daily feed-consumption data for Weeks 7 and 13, the average daily consumption of PETN calculated for this review was 109, 302.6, 362.5, 925, or 2,140 mg/kg-day, respectively, for males and 172, 306.3, 632.5, 1,340, or 3,120 mg/kg-day, respectively, for females. Clinical signs, body weights, feed consumption, and whole-blood MetHb concentrations were evaluated as reported above for rats. Necropsies and measurements of brain, heart, right kidney, liver, lungs, and thymus weights were performed on all animals. Comprehensive histological examinations were conducted in the control and highest dose groups of both sexes; histological evaluations at lower doses were limited to the liver in females at 1,340 mg/kg-day.

No mortalities, clinical signs of toxicity, or body-weight effects were reported. MetHb levels were <1% in all control and exposed groups. In highest dosed females, relative liver and kidney weights were slightly, but significantly, increased by 7−8%, compared with controls (see Table B-4); absolute organ weights were not reported. No additional organ-weight effects were noted (see Table B-4). No exposure-related gross or histopathological lesions were observed. A hepatocellular adenoma was observed in one female mouse at 3,120 mg/kg-day.

The highest dose used is a NOAEL of 3,120 mg/kg-day in females, identified for the lack of dose-related toxicologically relevant effects. The minor increases in relative liver and kidney weights in females are not considered toxicologically relevant effects due to their small magnitude (<10%).

Chronic/Carcinogenicity Studies

Bucher et al. (1990); NTP (1989) (Rat Study)

F344/N rats (50/sex/dose) were fed diets containing PETN NF for 2 years. Males were fed dietary concentrations of 0, 25,000, or 50,000 ppm PETN NF (0, 5,000, or 10,000 ppm PETN) and females were fed dietary concentrations of 0, 6,200, or 12,500 ppm (0, 1,240, or 2,500 ppm PETN). The lower dietary concentrations of PETN NF were selected for the female rats because higher concentrations caused 12−18% decreases in body-weight gains in the 14-week study summarized above. Bucher et al. (1990) reported an average daily PETN consumption of approximately 240 or 490 mg/kg-day in males and 80 or 165 mg/kg-day in females, respectively. Clinical signs, body weights, and feed consumption were evaluated throughout the study. Necropsies were performed on each rat. Comprehensive histological

examinations were performed on low-dose rats that died before Month 21 and on all control and high-dose rats. Histological examinations in the remaining low-dose rats were limited to the liver, kidneys, and gross lesions in both sexes; the brain, pancreas, and testes in males; and the esophagus, lungs, thyroid, and uterus in females.

No exposure-related changes were observed in survival, clinical signs, or feed consumption. Mean body weights were 2−9% lower in high-dose males throughout the study, compared with controls; terminal body weights were 7% lower than controls (statistics not reported; data reporting was inadequate for independent statistical review). Mean body weights for the low-dose males and all dosed females remained within 5% of control values throughout the study. No exposure-related non-neoplastic lesions were observed in either sex.

Adenomas or carcinomas of the Zymbal gland occurred in all chronically treated groups of male and female rats, but the incidences were low and did not demonstrate statistical significance when compared with controls. However, females had a significant dose-related trend in adenomas or carcinomas (see Table B-5). The incidences of Zymbal gland neoplasms exceeded the mean historical incidences for each sex, (see Table B-5). There were no increases in hyperplasia to suggest an increase of proliferative lesions of the Zymbal gland. Based on the occurrence of 9% Zymbal gland neoplasms in the high-dose female rats compared with none in controls, a statistically significant trend in females, (p = 0.028), and the occurrence of a Zymbal gland tumor in one high-dose female rat, (1/10 compared with 0/10 in controls) in the 14-week study summarized above, the study authors concluded that the results of the chronic study suggested a possible PETN-related effect.

Thyroid gland follicular cell adenomas or carcinomas (combined) were observed only in high-dose female rats (3/50). Although this incidence was not significantly increased compared with controls (0/50), there was a statistically significant dose-related trend in females and it exceeded historical control incidences (see Table B-5). Because there were no indications of increased follicular cell adenomas or carcinomas, or increased follicular cell hyperplasia in males, the NTP study authors did not consider the marginal increase in follicular cell tumors in females to be related to PETN. However, for the purposes of this PPRTV assessment, the U.S. EPA considers these thyroid tumors to be treatment related.

Other neoplastic findings included mononuclear leukemia in male rats that occurred with a negative trend due to an incidence in the high-dose group that was significantly lower than in controls.

A NOAEL of 10,000 ppm (490 mg/kg-day) is identified in male rats based on a lack of exposure-related noncancer effects. The body-weight decreases observed in high-dose males are not considered toxicologically relevant due to the small magnitude of effect (<10%). NTP concluded that there was “Equivocal Evidence of Carcinogenic Activity” of PETN in both male and female rats based on the increase in neoplasms of the Zymbal gland exceeding historical controls.

Bucher et al. (1990); NTP (1989) (Mouse Study)

B6C3F1 mice (50/sex/dose) were fed diets containing 0, 25,000, or 50,000 ppm PETN NF (0, 5,000, or 10,000 ppm PETN) for 2 years. Bucher et al. (1990) reported an average daily PETN consumption of approximately 810 or 1,620 mg/kg-day in males and 1,020 or 1,936 mg/kg-day in females. Clinical signs, body weights, and feed consumption were evaluated

throughout the study. Necropsy was performed on each mouse. Comprehensive histological examinations were performed on low-dose mice that died before Month 21 and on all control and high-dose mice. Histological examinations in the remaining low-dose mice were limited to the stomach and gross lesions in both sexes, and the liver and spleen in females.

There were no exposure-related decreases in survival, clinical signs, effects on body weight or food consumption, or increases in non-neoplastic or neoplastic lesions. Combined tumors of the subcutaneous tissues (primarily fibromas and fibrosarcomas) occurred with a negative dose-related trend in male mice; incidences in both dosed groups were significantly lower than in controls. Skin tumors in the male mice control group occurred at a rate nearly five times higher than in historical controls, but the study authors did not provide a rationale for this discrepancy.

For non-neoplastic effects, a NOAEL of 1,936 mg/kg-day in female mice is identified based on a lack of exposure-related effects. There was no evidence of carcinogenicity in male or female mice under the conditions of this bioassay.

Donahue (1944)

Male and female albino rats (45/group; sex ratio not reported) were fed PETN at dietary doses of 0 or 2 mg/kg-day for 1 year. Body weights were recorded weekly. Blood was collected monthly from 20 rats/group for hematology (erythrocyte count, hemoglobin [Hb] concentration total, and differential leukocyte counts). After the exposure period, the surviving animals were sacrificed. The liver, kidneys, spleen, heart with lungs, brain, and testes were removed, and the weight, volume, and density (ratio of weight to volume) were recorded. Histopathological examinations were performed on the liver, kidneys, spleen, heart, lungs, brain, and femur; the study authors noted that particular attention was paid to histological changes in the vascular walls of these tissues. Organs from animals that died before the scheduled sacrifice do not seem to have been examined.

High mortality (46.6% in the control group and 20% in the exposed group) was attributed to an infestation of parasitic tapeworm larvae observed in the livers of a large number of surviving rats (11/24 surviving controls, 12/36 surviving exposed). However, no data were provided regarding the presence or absence of parasitic infection in animals that died before the scheduled sacrifice. Body weights and growth curves were comparable between groups throughout the study. Erythrocyte and Hb values fluctuated throughout the study but remained within normal ranges for both control and exposed rats. Leukocyte values were abnormally high in both groups; the study authors attributed this finding to the observed parasitic infection. There were no exposure-related changes in organ weights, volumes, or density. No exposure-related histopathological lesions were observed.

A NOAEL or LOAEL are not identified from this study because confidence in this study is low due to the observed parasitic infestation and high mortality in both the control and exposure groups.

Reproductive/Developmental Studies

Quinn et al. (2009)

Groups of Sprague Dawley rats (10/sex/group) were administered PETN (purity >98%) at daily doses of 0, 100, 500, or 1,000 mg/kg-day via gavage in corn oil for up to 56 days. Dosing began 2 weeks prior to mating and continued in both sexes during mating. After mating,

dosing of males continued until a total dosing period of 28 days was completed, whereupon they were sacrificed. Female exposure continued through gestation until Postnatal Day (PND) 3; dams with offspring were sacrificed on PND 4. During the exposure period, the animals were observed daily for mortality and clinical signs of toxicity. Parental body weights were recorded weekly. During premating, pregnancy, and lactation, food consumption was measured weekly. During the mating period, pregnancy was indicated by presence of a sperm plug. Dams were allowed to deliver, and the litters were examined for the number and sex of pups, number of live and dead pups, number of runts, and presence of gross abnormalities. Live pups were counted and sexed, and litter weights were recorded on PNDs 1 and 4. At sacrifice, all parental animals and offspring were examined grossly. The testes and epididymides of all male parental animals were removed and weighed. The ovaries, testes, epididymides, and all organs showing macroscopic lesions from parental animals were examined microscopically.

No exposure-related mortalities were observed; four animals died during the exposure period due to gavage error (esophageal perforation). No clinical signs of toxicity were observed. Male body weights and food consumption were comparable between the exposure and control groups throughout the study. Body weights were significantly elevated by 3−9% in females exposed to 100 or 500 mg/kg-day on Day 20 of exposure (approximately the end of the mating period) and at necropsy, compared with control; female body weights were comparable to control at the high dose throughout the study. Increased body weights in females at 100 and 500 mg/kg-day were accompanied by significant 43−57% increases in food consumption during premating and pregnancy.

The study authors did not report mating or fertility indices, and the total number of pregnant females and litters produced per group was not reported. No changes in gestation duration, number of pups, or pup sex ratio were observed. On PND 1, male pup body weights were significantly elevated by 6% at 500 mg/kg-day; however, body weights were comparable to control at 100 and 1,000 mg/kg-day on PND 1 and in all dose groups on PND 4. No exposure-related changes were observed in female pup body weights. No exposure-related gross deformities or lesions were observed in offspring, but traditional microscopic developmental outcome analyses in pups were not performed.

In parental animals, there were no exposure-related changes in relative testes, epididymides, or ovary weights (absolute weights not reported). No gross lesions were observed in adult males or females at necropsy, and no exposure-related histological lesions were observed in the testes, epididymides, or ovaries. The numbers of corpora lutea and implants were comparable between control and exposed females.

A NOAEL of 1,000 mg/kg-day is identified based on a lack of toxicologically relevant effects in reproductive organs in parental animals, litter parameters, or neonatal pup body weight, survival, or gross morphology.

2.2.2. Inhalation Exposures

No repeated-exposure inhalation studies have been identified.

2.3. OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

2.3.1. Genotoxicity

Table 4 provides an overview of genotoxicity studies of PETN. “Military-grade” PETN solutions in dimethylsulfoxide (DMSO) did not induce reverse mutations in *Salmonella*

typhimurium when tested without metabolic activation in a spot test, or with metabolic activation in a plate incorporation assay (Whong et al., 1980). Similarly, the pharmaceutical-grade 1:4 PETN-lactose mixture used in the NTP toxicity and carcinogenicity studies (PETN NF) was not mutagenic in *S. typhimurium* when tested with or without metabolic activation in a preincubation assay (NTP, 1989; Mortelmans et al., 1986).

PETN NF induced a 17−31% increase in sister chromatid exchanges (SCEs) in cultured Chinese hamster ovary (CHO) cells in the presence and absence of metabolic activation; however, the response was not dose related and cell cycle delay was not induced (NTP, 1989). The lack of a dose-response may have been due to precipitation of the test compound at mid- and high-dose levels (NTP, 1989). PETN NF did not induce chromosomal aberrations (CAs) in CHO cells when tested with or without metabolic activation (NTP, 1989).

Table 4. Summary of PETN Genotoxicity

Endpoint	Test System	Doses/Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies in prokaryotic organisms						
Mutagenicity	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	0, 0.625, 1.25 mg/spot	−	ND	Spot test; test substance was military-grade PETN.	Whong et al. (1980)
Mutagenicity	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	Up to 2.5 mg/plate	ND	−	Plate incorporation assay; test substance was military-grade PETN.	Whong et al. (1980)
Mutagenicity	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	0, 100, 333, 1,000, 3,333, 10,000 µg/plate	−	−	Preincubation assay; test substance was pharmaceutical-grade PETN NF (1:4 PETN-lactose mixture); precipitate was noted at 10,000 µg/plate.	NTP (1989); Mortelmans et al. (1986)
Genotoxicity studies in mammalian cells—in vitro						
SCE	CHO cells	0, 160, 500, 1,600, 2,500 µg/mL	+	+	Test substance was pharmaceutical-grade PETN NF (1:4 PETN-lactose mixture). All concentrations induced a 17−31% increase in SCEs with and without metabolic activation, compared with controls; however, the response was not dose related and cell cycle delay was not induced. Precipitate was noted at ≥500 µg/plate.	NTP (1989)
CA	CHO cells	0, 1,000, 1,600, 2,500 µg/mL	−	−	Test substance was pharmaceutical-grade PETN NF (1:4 PETN-lactose mixture). CAs were not induced in the presence or absence of S9 activation.	NTP (1989)

[a]+ = positive; − = negative; ND = no data.

CA = chromosomal aberration; CHO = Chinese hamster ovary (cell line cells); PETN = pentaerythritol tetranitrate; PETN NF = National Formulary (NF) Grade PETN; SCE = sister chromatid exchange.

2.3.2. Additional Animal Studies

Acute Toxicity Studies

Several acute animal studies have evaluated cardiovascular endpoints following exposure to PETN. Statistically significant coronary artery dilation was observed in dogs following acute oral exposure to 9−12 mg/kg. Daily administration for 5 days did not result in nitrate tolerance (Fink and Bassenge, 1997; Bassenge et al., 1996). Observed effects in dogs exposed to lower doses (~0.4−0.8 mg/kg) included decreased coronary blood flow and reduced cardiac work load (Sullivan et al., 1964). Transient decreases in systemic blood pressure were also observed in most studies in rabbits, cats, and dogs following single oral exposures to 0.4−50 mg/kg (Mullenheim et al., 2001; Commarato et al., 1973; Banerjee et al., 1970; Sullivan et al., 1964; von Oettingen and Donahue, 1944); however, mean arterial pressure was not significantly decreased in dogs following exposure to 12 mg/kg (Bassenge et al., 1996). Decreased pulmonary arterial pressure, as well as bronchodilation, has also been reported in dogs following inhalation of low aerosol levels ranging from 11−225 µg; however, systemic cardiovascular changes (e.g., decreased heart rate, decreased systemic blood pressure) were not observed (Aviado et al., 1969).

Acute studies evaluating endpoints other than cardiovascular are limited to a single case study in a dog that ingested an unknown quantity of PETN while training to detect explosives (Potocnjak et al., 2008). The dog presented with central nervous system (CNS) depression, including bradycardia, swaying gait and broad-based stance, proprioceptive deficits in hind limbs, ataxia, inability to stand, and disorientation. Clinical tests showed transient mild anemia, increased urinary bilirubin, and increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT). The dog fully recovered after 1 week.

Studies Evaluating Actions of PETN in Animal Models of Cardiovascular Disease

Additional actions of PETN on the cardiovascular system have been studied using animal models of cardiovascular disease, including atherosclerosis (in cholesterol-fed rabbits) and hypertension (in N omega-nitro-L-arginine methyl ester [L-NAME] spontaneously hypertensive rats).

Studies in atherosclerotic rabbit models have suggested that PETN treatment may slow or prevent the formation of atherosclerotic lesions. In cholesterol-fed rabbits, dietary exposure to 6 mg/kg-day PETN for 15 weeks protected against the development of aortic atherosclerosis and endothelial dysfunction without affecting the vasodilatory potency of PETN in aortic rings (Kojda and Noack, 1995; Kojda et al., 1995). The results of a subsequent study in cholesterol-fed rabbits indicated that dietary exposure to 6 mg/kg-day PETN for 16 weeks reduced the progression of aortic lesion formation, endothelial dysfunction, and low-density lipoprotein (LDL) oxidation in established atherosclerosis (Hacker et al., 2001). Kojda et al. (1998) proposed that the observed reductions in vascular superoxide production following exposure to 6 mg/kg-day PETN for 16 weeks contributes to the observed protective effects of PETN in experimental atherosclerosis.

Evidence for therapeutic benefits of PETN treatment in hypertensive rat models are inconsistent. In spontaneously hypertensive rats, acute exposure to 30 mg/kg resulted in a statistically significant decrease in systolic blood pressure 15 minutes after administration, with blood pressure values returning to near-control levels by 30 minutes after administration (Commarato et al., 1973). Similarly, studies in hypertensive Wistar rats found that exposure to

PETN at twice daily doses of 50 mg/kg for 6 weeks resulted in a statistically significant 5% decrease in systolic blood pressure (Torok and Kristek, 2002; Kristek, 2000). Significant alterations in cardiovascular system parameters by PETN included reduced wall thickness of the thoracic aorta, carotid artery, and septal branch of the left descending coronary artery; reduced cross-sectional area of the carotid artery; increased inner diameter of the thoracic artery; and decreased wall: diameter ratio in the thoracic and carotid arteries. In contrast, subchronic studies in spontaneously hypertensive rats did not find any changes in general cardiovascular system parameters (including blood pressure or the geometry of conduit arteries, including inner diameter, wall thickness, or cross-sectional area of the thoracic aorta, carotid artery, and coronary artery) following exposure to PETN at once or twice daily doses of 100 mg/kg via gavage for 6 weeks (Dovinová et al., 2009; Gerová et al., 2005). However, Kristek et al. (2003) reported increased blood platelet cyclic guanosine 3c,5c-monophosphate (cGMP) content and decreased aortic nitric oxide synthase (NOS) activity.

While evidence for reduced blood pressure in hypertensive rats following PETN exposure is inconsistent, limited evidence suggests that pre- and postnatal exposure to PETN may have lasting effects on blood pressure and endothelial function. Following maternal exposure to 50 mg/kg-day during pregnancy and lactation, female offspring of spontaneously hypertensive rats show a persistent reduction in blood pressure in adulthood along with enhanced NO-mediated vasodilation in response to acetylcholine (Wu et al., 2015). No changes in blood pressure were observed in dams or male offspring.

2.3.3. Metabolism/Toxicokinetic Studies

Absorption and Distribution

In humans, oral absorption of PETN is relatively rapid. Davidson et al. (1970) and Davidson et al. (1971) identified radioactivity in the blood within 15 minutes of oral administration of radiolabeled PETN in humans, with peak blood radioactivity between 2−8 hours. Blood levels declined to about 40 and 10% of the peak level at 24 and 48 hours, respectively, with half-lives estimated in the range of 7.1−8.3 hours (Davidson et al., 1971). In another study, peak blood levels were achieved between 2 and 3 hours, with a plasma elimination half-life of 4−5 hours (Weber et al., 1995). Based on data in ligated rats, PETN is absorbed very slowly in the stomach, with increased rate of absorption in the intestines (DiCarlo et al., 1967). Initially (within 2 hours of administration), absorption is more rapid in the small intestine than the large intestine; however, the rate and extent of absorption in the large intestine increases between 2 and 4 hours. This shift in absorption is attributed to the extensive degradation of PETN into denitrated metabolites by bacterial flora in the large intestine; very little breakdown of PETN was observed in the stomach and small intestine (DiCarlo et al., 1967). Consistent with human data, maximal levels of radioactivity in the blood occur in rodents within 4 hours of oral administration of radiolabeled PETN [reviewed by Litchfield (1971)]. After absorption, PETN binds to both plasma proteins and erythrocytes, and is rapidly distributed throughout the body, with organ radioactivity levels greater than blood levels after 1 hour [reviewed by NTP (1989); Litchfield (1971)]. Absorption and distribution data are consistent with observed therapeutic effects, with peak vasodilatory effects of organic nitrates occurring 60−90 minutes following oral administration, with a duration of action of 3−6 hours [reviewed by Murad (1990)].

Metabolism

The metabolism of PETN has been extensively studied; a summary of available data is presented below based on the following reviews: Daiber et al. (2008), Daiber et al. (2004),

Daiber and Münzel (2015), Gori and Daiber (2009), Münzel et al. (2013), Klemenska and Beresewicz (2009), NTP (1989), Litchfield (1971), Murad (1990), and Münzel and Gori (2013). PETN is sequentially denitrated to form pentaerythritol trinitrate (PETriN, CASRN 1607-17-6), pentaerythritol dinitrate (PEDN, CASRN 1607-01-8), pentaerythritol mononitrate (PEMN, CASRN 1607-00-7), and pentaerythritol (PE, CASRN 115-77-5), releasing a molecule of inorganic nitrite at each denitration step. In humans, PEDN and PEMN are the primary compounds detected in blood following oral administration, with only trace amounts of PETriN; the parent compound has not been quantified in the blood. This may be due, in part, to the breakdown of PETN and PETriN by intestinal microorganisms before absorption. In vitro, PETN metabolism has been shown to occur in the blood (primarily in red blood cells [RBCs]), in subcellular fractions of the heart, by liver parenchymal and reticuloendothelial cells, and in isolated aortas. At low concentrations (<1 µM), PETN is sequentially denitrated by mitochondrial aldehyde dehydrogenase 2 (ALDH-2), and the nitrite molecules released during metabolism are reduced to NO in the mitochondria via various pathways. At higher concentrations, PETN and its denitrated metabolites can also be metabolized by cytochrome P450 (CYP450) in the smooth endoplasmic reticulum, leading to the generation of NO. Denitrated metabolites can be glucuronidated before excretion, and evidence from biliary cannulated rats indicated that glucuronidated metabolites can undergo enterohepatic circulation via reabsorption from the intestines after removal of glucuronic acid.

Excretion

Excretion is primarily via urine and feces, with negligible excretion of breakdown products via the lungs (Davidson et al., 1971; Litchfield, 1971; Davidson et al., 1970). In humans given a single 20-mg dose, mean urinary excretion was 53.1% of the administered dose after 24 hours and 60.3% after 48 hours, and mean fecal excretion was 31.5% of the administered dose after 48−72 hours (Davidson et al., 1970). Similar proportions were observed following a single 40-mg dose (49.8% of administered dose in urine after 48 hours and 41.2% of administered dose in feces after 72 hours) (Davidson et al., 1971). The primary compounds identified in urine were PEMN and PE, while PETN and PE were the primary compounds in feces (see Table 5) (Davidson et al., 1971, 1970). The presence of PETN in feces was attributed to unabsorbed and unmetabolized parent compound and/or excretion of PETN via enterohepatic circulation, while PE was attributed to metabolic breakdown of PETN by intestinal flora and/or excretion of PETN via enterohepatic circulation (Davidson et al., 1971, 1970). The renal excretion of primary metabolites PEMN and PE in humans was first order, with half-lives of 7.1 hours following a 20-mg dose and 8.3 hours following a 40-mg dose (Davidson et al., 1971). In rats or mice, 36% of the administered oral dose was excreted within 24 hours in the urine, with 60% of the administered dose recovered after 4 days; 10% of the administered dose was recovered in rat feces (Litchfield, 1971). The primary urinary metabolites were PEMN and PE in rats and PE in mice, and the primary fecal metabolite was PE (NTP, 1989; Litchfield, 1971). In dogs, 88% of the administered oral dose was excreted in urine, primarily as PEMN and PE, and 10% was excreted in feces, primarily as PE (DiCarlo et al., 1969).

Table 5. Excretion of PETN and Metabolites in Humans Following a Single Oral Exposure

Compound Excreted	20 mg[a]		40 mg[b]	
	Urine	Feces	Urine	Feces
Percent of administered dose recovered (as radioactivity) within 48 h (urine) or 48−72 h (feces)	60.3 ± 3.6	31.5 ± 4.8	49.8	41.2
Relative quantities (%) of parent compound and metabolites:				
PETN	None to trace[c]	26.7 ± 13.4	None	45
PETriN	None	0.4 ± 0.8	None	None
PEDN	0.6 ± 0.4	0.9 ± 1.5	1	2
PEMN	51.4 ± 10.3	5.9 ± 3.5	74	2
PE	48.1 ± 10.4	66.3 ± 14.6	25	51

[a]Davidson et al. (1970); data presented as mean ± SD, as reported by the study authors (percent excreted in urine and feces) or as calculated for this review based on individual subject data (relative quantities).
[b]Davidson et al. (1971); data presented as mean, as reported by the study authors (percent of administered dose recovered in urine and feces) or approximate mean (relative percent quantities of parent compound and metabolites; estimated from graphically presented data).
[c]PETN detected in trace amounts in the urine of one subject.

PE = pentaerythritol; PEDN = pentaerythritol dinitrate; PEMN = pentaerythritol mononitrate; PETN = pentaerythritol tetranitrate; PETriN = pentaerythritol trinitrate; SD = standard deviation.

2.3.4. Mode-of-Action/Mechanistic Studies

The therapeutic vasodilatory action of organic nitrates, including PETN, are attributed to their active intermediate, NO [reviewed by Daiber and Münzel (2015); Münzel et al. (2013); Kosmicki (2009); Daiber et al. (2008); Bode-Böger and Kojda (2005); Murad (1990)]. As discussed in the "Metabolism" section above, NO is released during PETN metabolism. Free NO is transported into the nucleus of vascular smooth muscle cells where it initiates the NO/cGMP intracellular signaling pathway, ultimately leading to smooth muscle relaxation [reviewed by Daiber and Münzel (2015); Münzel et al. (2013); Münzel and Gori (2013); Klemenska and Beresewicz (2009); Daiber et al. (2008); Murad (1990)]. However, the mechanisms underlying cerebral vasodilation (leading to the primary toxicologically relevant side effect of headaches) seem to be different from those identified for coronary vasodilation (therapeutic goal). As reviewed by Bode-Böger and Kojda (2005), the 100-fold decrease in the degree of vasodilation in cerebral arteries versus coronary arteries, in response to organic nitrate therapy, along with the cessation of headache after the first few days of therapy, supports the hypothesis that cerebral vasodilation occurs via a different pathway than coronary vasodilation. Evidence suggests that cerebral arteries may lack enzymes required for bioactivation of nitrates to NO. One proposed alternate mechanism for cerebral vasodilation in the absence of metabolism-generated NO is activation of the NO/cGMP pathway subsequent to direct activation of sensory nerve fibers by organic nitrates, triggering a release of calcitonin gene-related peptide.

Long-term organic nitrate use is generally associated with nitrate tolerance, endothelial dysfunction, sympathetic activation, and a potential increase in risk for ischemic episodes. PETN, however, is unique among the long-acting nitrovasodilators because chronic use is not associated with these effects. Long-acting in this instance refers to the action of the drug compared with NTG, not to the extended-release formulation of PETN, which is a separate issue. The mechanisms of nitrate tolerance have been extensively researched, and a summary of

available data is presented below based on the following reviews: Bai et al. (2018); Opelt et al. (2018); Steven et al. (2017); Daiber and Münzel (2015); Münzel et al. (2013); Münzel and Gori (2013); Rutherford and Struthers (2013); Daiber et al. (2012); Daiber and Münzel (2010); Daiber et al. (2009); Klemenska and Beresewicz (2009); Kosmicki (2009); Daiber et al. (2008); Daiber et al. (2004). There are several potential mechanisms for nitrate tolerance and endothelial dysfunction following extended nitrate therapy, such as with NTG. Release of NO from PETN by ALDH-2 results in oxidation of the enzyme at the reactive cysteine C302, which requires an endogenous reductant for reactivation, which can be depleted, leading to the formation of reactive oxygen species (ROS). ROS generation and the subsequent formation of superoxide or peroxynitrite, can lead to reversible or irreversible inactivation of ALDH-2, resulting in reduced NO generation and a decreased therapeutic effect following nitrate administration. Additionally, peroxynitrite can cause uncoupling of endothelial NOS, leading to endothelial dysfunction. An additional mechanism of endothelial dysfunction and nitrate resistance may be through interaction of nitrates with the expression of prostaglandin I2 (PGI2) synthase. Nitrate donors induce the expression of miR-199 (a micro-RNA), which targets the PGI2 synthase messenger RNA (mRNA), lowering PGI2 levels and blocking vasodilation through that mechanism (Bai et al., 2018). However, neither PETN nor its metabolite PETriN affects the nitrate esterase activity of ALDH-2 or elicits ROS formation in isolated arteries or mitochondria. Furthermore, PETN exhibits intrinsic antioxidant properties due to the redox potential of its dinitrate metabolite and induction of protective genes, both in vitro and in vivo, including heme-oxygenase (HO-1) and ferritin (HO-1 mediates the conversion of heme into bilirubin, one of the strongest antioxidants in the body, and ferritin chelates iron, which suppresses hydroxyl radical formation). These antioxidant actions of PETN may explain the lack of nitrate tolerance or endothelial dysfunction following PETN therapy.

3. DERIVATION OF PROVISIONAL VALUES

3.1. DERIVATION OF ORAL REFERENCE DOSES

3.1.1. Derivation of a Subchronic Provisional Reference Dose

The database of potentially relevant studies for deriving a subchronic provisional reference dose (p-RfD) for PETN includes numerous clinical studies evaluating the compound's therapeutic use as a venous dilator at doses ranging from 0.57−4.6 mg/kg-day for the long-term treatment of cardiovascular diseases (see Table 3A). In addition, several acute studies have established a single-exposure effective dose for reduced blood pressure as low as 0.29 mg/kg (Dagenais et al., 1969). The available animal studies are considered less relevant for deriving the subchronic p-RfD due to the extent of the human database and the much higher doses (39.1−3,120 mg/kg-day) evaluated in animal studies, including a subchronic-duration dietary study in mice and rats (Bucher et al., 1990; NTP, 1989) and a reproductive/developmental (R/D) gavage study in rats (Quinn et al., 2009). Effects associated with the therapeutic use of organic nitrates are well characterized and generally secondary to actions on the cardiovascular system, including hypotension, headache, and dizziness due to cerebral vasodilation, along with other subjective complaints [reviewed by Daiber et al. (2008); Bode-Böger and Kojda (2005); Murad (1990)]. As discussed in the "Human Studies" section and shown in Table 3A, several of the available clinical trials reported an increase in toxicologically relevant side effects following exposure to PETN.

The designation of the POD for the subchronic p-RfD for PETN is focused on identifying the lowest therapeutic dose because the primary effect of vasodilation is considered to be toxicologically relevant in the general population, particularly in chronically exposed individuals with pre-existing hypotension or susceptible to hypotension; such people include pregnant women, infants, the elderly suffering from dehydration or malnutrition, diabetics, and those taking certain medications (diuretics, antidepressants, erectile dysfunction drugs). The lowest dose of PETN associated with a vasodilatory effect in the continuous-exposure clinical trials is 0.57 mg/kg-day in the Phillips (1953) study. This study, however, involved intermittent concurrent treatment with NTG, which elicits the same effects as PETN, thereby making the determination of the specific effective dose of PETN difficult. Because PETN and NTG act in the same way, though NTG has a shorter half-life, the combined therapeutically effective dose will be higher than for either drug alone. This study, and several others with concurrent NTG treatment (Cass and Cohen, 1961; Plotz, 1960; Rosenberg and Michelson, 1955) were not considered further as the basis for the POD. Of the remaining PETN-only, continuous-exposure studies, the lowest effective vasodilatory dose of PETN was 0.86 mg/kg-day (Hedges and Gordon, 1965). Note that the most of the studies mentioned above with concurrent NTG treatment (Cass and Cohen, 1961; Plotz, 1960; Rosenberg and Michelson, 1955) also showed the same lowest effective dose at 0.86 mg/kg-day. In those studies, NTG treatment was episodic, and thus may not have consistently influenced the effective dose of PETN. Those studies are thus supportive of using the 0.86 mg/kg-day LOAEL as a potential POD. Vasodilation was implicit in Hedges and Gordon (1965), and in most of the other clinical trials, and was judged to be present based on the observed therapeutic efficacy at the treatment dose. Other clinical studies reported effective continuous treatment results at doses ranging from 50 mg (Bohm and Haustein, 1998) to 80 mg (Edson et al., 1961) and higher (Schleussner et al., 2014; Schnorbus et al., 2010; Jurt et al., 2001; Shrivastava et al., 1983; Aubert et al., 1970). The effective therapeutic dose for all these studies was the lowest (or only) administered treatment level. No clear NOAELs for therapeutic efficacy were reported in the continuous-exposure studies. Rosenberg and Michelson

(1955) briefly mentioned a lack of therapeutic efficacy at a dose of 0.86 mg/kg-day for five patients in a preliminary trial to their main study. This study, however, was discounted because the study was confounded by concurrent treatment with NTG.

NOAELs for side effects in the continuous-exposure clinical studies have been established in placebo-controlled studies, without concurrent NTG treatment, at doses as low as 0.86 mg/kg-day in the relevant (not confounded by NTG treatment) continuous exposure studies for headaches in 3/72 patients (Hedges and Gordon, 1965). Evidence from the placebo-controlled studies of greater than 4 weeks showed NOAELs for side effects ranging from 0.57−2.3 mg/kg-day (Hedges and Gordon, 1965; Plotz, 1960; Rosenberg and Michelson, 1955; Phillips, 1953).

Several acute-exposure (single-dose) studies reported effective doses of PETN; these doses ranged from 0.29−1.1 mg/kg (Dragoni et al., 2007; Bohm and Haustein, 1998; Giles et al., 1981; Amsterdam et al., 1980; Shellock et al., 1980; Dagenais et al., 1969). The lowest effective dose from a single exposure (0.29 mg/kg) was reported by Dagenais et al. (1969) to be associated with a 5−10% reduction in systolic blood pressure in 15 angina patients; this result by itself, would seem to define the most sensitive effect, which, in this case, was an overt reduction in blood pressure, rather than vasodilation.

Conclusion

Environmental exposures to PETN, as outlined previously, could lead to toxicologically relevant vasodilatory effects in the general healthy population, but particularly in chronically exposed individuals with pre-existing hypotension or those susceptible to hypotension, such as pregnant women, infants, the elderly suffering from dehydration or malnutrition, those with diabetes, and those taking certain medications (diuretics, antidepressants, erectile dysfunction drugs). Thus, the level of potential concern for the general population would be the lowest effective therapeutic dose.

Approach for Deriving the Subchronic p-RfD

The lowest LOAEL applicable to continuous exposure of PETN is (0.86 mg/kg-day) from the continuous-exposure clinical study of Hedges and Gordon (1965). Some single-dose studies had lower effect levels between 0.29−0.71 mg/kg (Bohm and Haustein, 1998; Giles et al., 1981; Amsterdam et al., 1980; Dagenais et al., 1969). However, given the uncertainties in extrapolating an acute exposure to an equivalent continuous exposure, effect levels derived from the repeat-exposure clinical studies are judged to be much more representative of continuous daily human exposures. Therefore, the single-dose acute studies are not considered further as the basis for the subchronic p-RfD.

A LOAEL of 0.86 mg/kg-day is selected as the POD for deriving the subchronic p-RfD based on implicit vasodilation, given the therapeutic efficacy of PETN at that average daily dose (ADD) administered to coronary heart disease patients (Hedges and Gordon, 1965). Angina was reduced by more than two-thirds by patients taking PETN compared with control. Some patients also left the study because of side effects of lowered blood pressure, again indicating therapeutic efficacy. The ADD was estimated from the treatment schedule of one 30-mg extended-release tablet every 12 hours, assuming a 70 kg adult body weight. The 0.86-mg/kg-day dose level was the lowest treatment dose; a NOAEL was not established in this study. Although the treatment protocol included a 2-week placebo administration phase intervening between the two 2-week PETN treatment phases, averaging the effective PETN dose over the entire 6 weeks was judged

to be inappropriate. The latter decision is based on the reported efficacy of treatment during the first 2 weeks of PETN administration (Hedges and Gordon, 1965) and immediate biological action reported following single doses of 20−40 mg PETN (Amsterdam et al., 1980; Dagenais et al., 1969). These observations are consistent with the short half-life and duration of efficacy for PETN, both on the order of a few hours (Weber et al., 1995). In addition, the LOAEL of 0.86 mg/kg-day may be near a threshold, given the observations of no effect of PETN in a preliminary study (Rosenberg and Michelson, 1955), but with effects at the same dose in additional placebo-controlled continuous studies 4 weeks in duration or longer (Cass and Cohen, 1961; Plotz, 1960; Roberts, 1958). Thus, there is evidence across the database that NOAELs and LOAELs are overlapping. All these considerations indicate that the biological activity of PETN depends more on the immediate (peak) internal concentration than on a longer-term average.

Therefore, although the LOAEL POD is based on a relatively short exposure duration, the effects are expected to be due to the acute vasodilatory effects of PETN, and longer-term exposure would not be required to evaluate efficacy. In those studies that used the extended-release formulation, the internal exposure would be somewhat continuous. As for the potential for increased toxicity with longer exposures, few side effects were evident at exposures up to 30 weeks. The side effects were generally mild, consisting of headaches, dizziness, and nausea, all attributable to the primary effect of vasodilation. Furthermore, significantly higher doses in subchronic-duration animal studies reveal no toxicologically relevant effects with a LOAEL of 200 mg/kg-day in rats, and a NOAEL of 3,120 mg/kg-day in mice (Bucher et al., 1990; NTP, 1989). Therefore, the lowest effective therapeutic dose in evidence from the large clinical study database is deemed appropriate as the basis for a LOAEL in the general human population and to serve as a POD for the subchronic p-RfD. The subchronic p-RfD for PETN, based on the LOAEL of 0.86 mg/kg-day (Hedges and Gordon, 1965), is supported by two additional studies (Cass and Cohen, 1961; Plotz, 1960); however, these two studies were partially confounded by concurrent treatment with NTG. Thus, the subchronic p-RfD is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= \text{LOAEL} \div UF_C \\ &= 0.86 \text{ mg/kg-day} \div 100 \\ &= \mathbf{9 \times 10^{-3} \text{ mg/kg-day}} \end{aligned}$$

Table 6 summarizes the uncertainty factors for the subchronic p-RfD for PETN.

Table 6. Uncertainty Factors for the Subchronic p-RfD for PETN

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied because the assessment is based on clinical data from humans.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied. The database contains numerous clinical oral studies, including several recent, medium to large, well-designed, placebo-controlled, double-blind, randomized studies of 4−30 wk in duration, that identify NOAELs or LOAELs in cardiovascular disease patients treated with PETN to induce coronary vasodilation at doses up to 4.6 mg/kg-d. The database also includes short-term-, subchronic-, and chronic-duration oral studies in rats and mice that found either no effects or only mild nonspecific effects on body weight at high doses (≥200 mg/kg-d) (Bucher et al., 1990; NTP, 1989), as well as an oral R/D toxicity screening study in rats that found no effects at doses up to 1,000 mg/kg-d (Quinn et al., 2009). However, a multigeneration reproductive toxicity study or a developmental teratology study have not been conducted.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility (including hypotensive individuals) in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of PETN in humans.
UF_L	3	A UF_L of 3 ($10^{0.5}$) is applied because of overlapping NOAEL and LOAEL values likely near the threshold response, for example, Rosenberg and Michelson (1955).
UF_S	1	UF_S is not applicable to the subchronic p-RfD because the principal study was 8 wk in duration and because the very short half-life of NO causes dosing to be episodic, not cumulative, and thus the effects would not be dependent on duration.
UF_C	100	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

LOAEL = lowest-observed-adverse-effect level; NO = nitric oxide; NOAEL = no-observed-adverse-effect level; PETN = pentaerythritol tetranitrate; p-RfD = provisional reference dose; R/D = reproductive/developmental; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Confidence in the subchronic p-RfD for PETN is medium as explained in Table 7.

Table 7. Confidence Descriptors for the Subchronic p-RfD for PETN

Confidence Categories	Designation	Discussion
Confidence in principal study	M	Confidence in the principal study (Hedges and Gordon, 1965) is medium. The study appears to be of high quality, with a single-blind randomized crossover design, 72 subjects, and clear, well-reported results. However, a NOAEL was not identified because effects were seen at both doses in the study.
Confidence in database	M	Confidence in the database is medium. The database comprises multiple well-conducted and well-reported clinical trials that demonstrate the effects of PETN in cardiac patients and healthy volunteers at exposure levels near the POD. The database also contains short-term-, subchronic-, and chronic-duration oral studies in rats and mice that found either no effects or only mild nonspecific effects on body weight at high doses (≥200 mg/kg-d) (Bucher et al., 1990; NTP, 1989), as well as an oral R/D toxicity screening study in rats that found no effects at doses up to 1,000 mg/kg-d (Quinn et al., 2009). However, neither a multigenerational reproductive study nor a developmental teratology study has been done.
Confidence in subchronic p-RfD[a]	M	Overall confidence in the subchronic p-RfD is medium.

[a]The overall confidence cannot be greater than lowest entry in table (medium).

M = medium; NOAEL = no-observed-adverse-effect level; PETN = pentaerythritol tetranitrate; POD = point of departure; p-RfD = provisional reference dose; R/D = reproductive/developmental.

3.1.2. Derivation of a Chronic Provisional Reference Dose

As discussed for the derivation of the subchronic p-RfD, the database of potentially relevant studies for deriving a chronic reference dose value for PETN includes numerous clinical studies evaluating the compound's therapeutic use as a venous dilator for the long-term treatment of cardiovascular diseases using therapeutic doses ranging from 0.57−4.6 mg/kg-day (see Table 3A). Due to the extent of the human database, the available animal studies evaluating much higher doses (39.1−3,120 mg/kg-day), including a chronic-duration dietary study in mice and rats (Bucher et al., 1990; NTP, 1989) and an R/D gavage study in rats (Quinn et al., 2009), were not considered as principal studies for deriving the chronic p-RfD. While no chronic-duration clinical studies were identified, any potential toxicologically relevant effects of PETN are expected to be due to acute vasodilatory effects of PETN and transient in nature. Further, the results of the chronic-duration animal studies revealed no additional noncancer hazards. Therefore, short-term-duration clinical studies are considered appropriate for deriving the chronic p-RfD.

Approach for Deriving the Chronic p-RfD

The basis for the chronic p-RfD is the same as for the subchronic p-RfD, with the lowest effective therapeutic dose serving as a LOAEL and vasodilation as the critical effect. The LOAEL is 0.86 mg/kg-day for vasodilation, with potential for reduced blood pressure, established in the clinical study of Hedges and Gordon (1965). The chronic p-RfD for PETN is derived as follows:

Chronic p-RfD = NOAEL ÷ UF_C
= 0.86 mg/kg-day ÷ 100
= **9×10^{-3} mg/kg-day**

Table 8 summarizes the uncertainty factors for the chronic p-RfD for PETN.

Table 8. Uncertainty Factors for the Chronic p-RfD for PETN

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied because the assessment is based on clinical data from humans.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied. The database contains numerous clinical oral studies, including several recent, medium to large, well-designed, placebo-controlled, double-blind, randomized studies of 4−30 wk in duration that identify NOAELs or LOAELs in cardiovascular disease patients treated with PETN to induce coronary vasodilation at doses up to 4.6 mg/kg-d. The database also includes short-term-, subchronic-, and chronic-duration oral studies in rats and mice that found either no effects or only mild nonspecific effects on body weight at high doses (≥200 mg/kg-d) (Bucher et al., 1990; NTP, 1989), as well as an oral R/D toxicity screening study in rats that found no effects at doses up to 1,000 mg/kg-d (Quinn et al., 2009). However, a multigeneration reproductive toxicity study or a developmental teratology study have not been conducted.
UF_H	10	A UF_H of 10 is applied to account for human-to-human variability in susceptibility (including hypotensive individuals) in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of PETN in humans.
UF_L	3	A UF_L of 3 ($10^{0.5}$) is applied because of overlapping NOEAL and LOAEL values likely near the threshold of response. For example, Rosenberg and Michelson (1955).
UF_S	1	A UF_S of 1 is applied. Although the assessment is based on relatively short 8−30-wk clinical trials in humans, any potential toxicologically relevant effects of PETN are expected to be due to the acute vasodilatory effects of PETN and transient in nature. Furthermore, the database included 2-yr studies in rats and mice that assessed systemic toxicity at doses much higher than the human clinical doses and reported no effects. Therefore, increased risk is not expected following longer duration exposure.
UF_C	100	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PETN = pentaerythritol tetranitrate; p-RfD = provisional reference dose; R/D = reproductive/developmental; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies variability uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

The confidence of the chronic p-RfD for PETN is medium as explained in Table 9.

Table 9. Confidence Descriptors for the Chronic p-RfD for PETN

Confidence Categories	Designation	Discussion
Confidence in the principal study	M	Confidence in the principal study (Hedges and Gordon, 1965) is medium. The study appears to be of high quality, with a single-blind randomized crossover design, 72 subjects, and clear, well-reported results. However, a NOAEL was not identified, because effects were observed at both doses used in the study.
Confidence in database	M	Confidence in the database is medium. The database comprises multiple well-conducted and well-reported clinical trials that demonstrate the effects of PETN in cardiac patients and healthy volunteers at exposure levels near the POD. The database also contains short-term-, subchronic-, and chronic-duration oral studies in rats and mice that found either no effects or only mild nonspecific effects on body weight at high doses (≥200 mg/kg-d) (Bucher et al., 1990; NTP, 1989), as well as an oral R/D toxicity screening study in rats that found no effects at doses up to 1,000 mg/kg-d (Quinn et al., 2009). However, neither a multigenerational reproductive study nor a developmental teratology study has been done.
Confidence in chronic p-RfD[a]	M	Overall confidence in the subchronic p-RfD is medium.

[a]The overall confidence cannot be greater than lowest entry in table (medium).

M = medium; NOAEL = no-observed-adverse-effect level; PETN = pentaerythritol tetranitrate; POD = point of departure; p-RfD = provisional reference dose; R/D = reproductive/developmental.

3.2. DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No information was available on the subchronic or chronic inhalation toxicity of PETN, thus precluding the derivation of provisional reference concentration (p-RfC) values for PETN.

3.3. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

A summary of the noncancer provisional reference values is shown in Table 10.

Table 10. Summary of Noncancer Reference Values for PETN (CASRN 78-11-5)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Human/ both	Vasodilation	9×10^{-3}	LOAEL	0.86	100	Hedges and Gordon (1965)[a]
Chronic p-RfD (mg/kg-d)	Human/ both	Vasodilation	9×10^{-3}	LOAEL	0.86	100	Hedges and Gordon (1965)[a]
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

[a]See Table 3A.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NDr = not determined; PETN = pentaerythritol tetranitrate; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

3.4. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

The cancer weight-of-evidence (WOE) descriptor for PETN is *"Suggestive Evidence of Carcinogenic Potential"* following oral exposure and *"Inadequate Information to Assess Carcinogenic Potential"* following inhalation exposure; see the details below and in Table 11.

Table 11. Cancer WOE Descriptors for PETN

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	No human data are available.
"Likely to Be Carcinogenic to Humans"	NS	NA	The available data do not support this descriptor.
"Suggestive Evidence of Carcinogenic Potential"	**Selected**	**Oral**	**There is suggestive evidence of Zymbal gland tumors in M and F rats and thyroid tumors in F rats in a 2-yr oral bioassay (Bucher et al., 1990; NTP, 1989). There is no evidence of carcinogenicity in M or F mice in a 2-yr oral bioassay (Bucher et al., 1990; NTP, 1989) (see Appendix A).**
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Inhalation**	**No carcinogenicity studies are available that evaluated inhalation exposure.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	The available data do not support this descriptor.

F = female(s); M = male(s); NA = not applicable; NS = not selected; PETN = pentaerythritol tetranitrate; WOE = weight of evidence.

Following U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, the database for exposure to PETN provides evidence leading to a WOE descriptor of *"Suggestive Evidence of Carcinogenic Potential"* following oral exposure, in that both rare (normal incidence 1% or less) Zymbal gland and thyroid gland tumors were identified. In a 2-year bioassay in rats, incidences of rare Zymbal gland carcinomas or adenomas were observed in all treated groups of both sexes (see Table B-5). The incidences did not reach statistical significance ($p < 0.05$) when compared with control group incidences. However, they are rare tumors (both concurrent control and historical control incidence is less than 1%) that did exceed mean historical incidences for each sex, and there was a statistically significant trend in females ($p < 0.028$) (see Table B-5). Site concordance between males and females and a dose-response in females also adds to the WOE. Considering the overall occurrence of rare Zymbal gland tumors in 3 of 35 (9%) of dosed female rats compared with none in the controls in the chronic study, and a Zymbal gland tumor in one high-dose female rat in the subchronic-duration 14-week study, NTP (1989) concluded that the Zymbal gland tumors were possibly related to PETN exposure. Additional neoplastic findings in the rats included thyroid gland follicular cell adenomas or carcinomas in a small number of high-dose females (see Table B-5). The high-dose incidence was not statistically significantly higher than that in controls, but the incidence exceeded historical control incidences and showed a statistically significant dose-related trend (Cochran-Armitage trend test, $p = 0.016$; see Table B-5). For the purposes of this PPRTV assessment, the U.S. EPA considers these thyroid tumors to be treatment related. Because there were no indications of increased thyroid follicular cell adenomas, carcinomas, or hyperplasia in chronically exposed males, the small increase in follicular cell tumors in females was not considered PETN-related by NTP; however, based on the increase in neoplasms of the Zymbal gland, the NTP study authors concluded that this study provided "Equivocal Evidence of Carcinogenic Activity" of PETN for male and female F344/N rats.

In this assessment, the study results show a rare tumor type (Zymbal gland tumors) with a statistically significant dose-response in females, but not males (Bucher et al., 1990; NTP, 1989). This tumor type also appears in a high-dose female in the subchronic (14-week) assay. There is also evidence of thyroid tumors, with a statistically significant trend in females. NTP designated the carcinogenic potential "Equivocal." U.S. EPA finds that the observation of rare Zymbal gland tumors in both female and male rats and thyroid tumors in female rats constitute sufficient evidence for the identification of a hazard, but not strong enough to warrant a descriptor of *"Likely to Be Carcinogenic to Humans."* Thus, for this assessment, the cancer descriptor for PETN is determined to be *"Suggestive Evidence of Carcinogenic Potential."*

3.5. MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005) defines mode of action (MOA) "…as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Examples of possible modes of carcinogenic action for any given chemical include "mutagenicity, mitogenesis, programmed cell death, cytotoxicity with reparative cell proliferation, and immune suppression."

A limited amount of information is available on the genotoxicity and mutagenicity of PETN. Available in vitro data indicate that PETN and its metabolites are not mutagenic in bacterial systems (NTP, 1989; Mortelmans et al., 1986; Whong et al., 1980). Limited in vitro data indicate that PETN has the potential to induce clastogenic effects in mammalian cells (NTP,

1989). No additional data regarding potential mechanisms of carcinogenicity are available. Thus, a detailed MOA discussion for PETN is precluded.

3.6. DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

3.6.1. Derivation of a Provisional Oral Slope Factor

Although there is sufficient data to derive a provisional oral slope factor (p-OSF), the U.S. EPA cancer guidelines (U.S. EPA, 2005) state that a quantitative assessment is not usually conducted when the cancer descriptor is *"Suggestive Evidence of Carcinogenic Potential."* However, they go on to say that, if a quantitative assessment is useful for a specific purpose, it may be conducted. Therefore, for purposes of this provisional value assessment, a screening p-OSF is presented in Appendix A.

3.6.2. Derivation of a Provisional Inhalation Unit Risk

The lack of data on the carcinogenicity of PETN following inhalation exposure precludes deriving a quantitatively estimated provisional inhalation unit risk (p-IUR).

3.6.3. Summary of Cancer Risk Estimates

A summary of the cancer risk estimates is shown in Table 12.

Table 12. Summary of Cancer Risk Estimates for PETN (CASRN 78-11-5)

Toxicity Type (units)	Species/Sex	Tumor Type	Cancer Risk Estimate	Principal Study
Screening p-OSF $(mg/kg\text{-}d)^{-1}$	F344/N rat, F	Combined Zymbal gland and thyroid (see Appendix A)	4.3×10^{-3}	Bucher et al. (1990); NTP (1989)
p-IUR $(mg/m^3)^{-1}$	NDr			

F = female(s); NDr = not determined; PETN = pentaerythritol tetranitrate; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. SCREENING PROVISIONAL VALUES

For the reasons noted in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) document, it is inappropriate to derive provisional cancer toxicity values for pentaerythritol tetranitrate (PETN). However, information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited 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 deriving 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.

A National Toxicology Program (NTP) 2-year bioassay in rats and mice is available for developing a screening provisional oral slope factor (p-OSF) (Bucher et al., 1990; NTP, 1989). In the rat study, "Equivocal Evidence of Carcinogenicity" was observed in female and male rats based on marginal increases in combined incidence of rare Zymbal gland adenomas and carcinomas. There was also a marginal increase in the combined incidence of thyroid gland follicular cell adenomas and carcinomas in female rats. Although the NTP (1989) concluded that thyroid gland tumors were not related to PETN because there were no statistical indications of increased follicular cell adenomas or carcinomas, or increased follicular cell hyperplasia in males, the U.S. EPA considers suggestive evidence from a single sex to be informative. In the mouse study, there was no evidence of carcinogenicity.

Benchmark dose (BMD) modeling was performed for Zymbal gland tumors in female rats (see Table A-1; additional BMD details in Appendix C). Rationale for modeling marginal increases for Zymbal gland tumors include a significant dose-related trend in females (see Table B-5) and evidence for potential biological relevance based on their presence in every group of chronically treated rats and one high-dose female in the companion subchronic study (Bucher et al., 1990; NTP, 1989). Thyroid gland tumors in females were also BMD modeled because there was a significant dose-related trend in these tumors.

Before modeling, all doses were converted to human equivalent doses (HEDs) using 3/4 body-weight ($BW^{3/4}$) scaling (U.S. EPA, 2005), according to the equation below:

$$HED = dose \times (BW_a \div BW_h)^{1/4}$$

where

Dose = average daily animal dose (ADD)
BW_a = study-specific, time-weighted, body weight averages (TWA) for rat (see Table 3B footnote)
BW_h = human body weight [70 kg; U.S. EPA (2011c)]

The animal doses, calculated HED values, and associated Zymbal gland tumor and thyroid tumor incidences are provided in Table A-1.

Table A-1. Animal Doses, HEDs, and Zymbal and Thyroid Gland Adenoma or Carcinoma Incidences in Female Rats Exposed Orally to PETN[a]

Animal Dose mg/kg-d	HED mg/kg-d	Zymbal Gland Adenoma or Carcinoma Incidence	Thyroid Gland Adenoma or Carcinoma Incidence
0	0	0/36	0/50
80	20	1/37	0/48
165	41.3	3/35	3/50

[a]Bucher et al. (1990); NTP (1989).

HED = human equivalent dose; PETN = pentaerythritol tetranitrate.

BMD modeling of the data on incidences of Zymbal and thyroid gland adenomas or carcinomas in female rats (Bucher et al., 1990; NTP, 1989) yielded the 10% benchmark dose lower confidence limit ($BMDL_{10}$) (HED) values shown in Table A-2. Modeling procedures and results are described in detail in Appendix C.

Table A-2. BMD Model Results for Zymbal and Thyroid Gland Tumors in Female Rats[a]

Reference	Tumor Endpoint	Model Type	Goodness-of-Fit *p*-Value	BMD_{10} (HED) mg/kg-d	$BMDL_{10}$ (HED) mg/kg-d	p-OSF $(mg/kg\text{-}d)^{-1}$
Bucher et al. (1990); NTP (1989)	Zymbal gland adenoma or carcinoma in female rats	Multistage-Cancer-1st degree	0.9224	56	27	3.7×10^{-3}
Bucher et al. (1990); NTP (1989)	Thyroid gland adenoma or carcinoma in female rats	Multistage-Cancer-2nd degree	0.7050	60	40	2.5×10^{-3}
Bucher et al. (1990); NTP (1989)	**Combined Zymbal adenoma or carcinoma, or thyroid adenoma or carcinoma**	**MS-Combo**	**NA**	**36**	**23**	**4.3×10^{-3}**

[a]Bucher et al. (1990); NTP (1989).

BMD = benchmark dose; BMDL = benchmark dose lower confidence limit (subscripts denote BMR: i.e., 10 = exposure concentration associated with 10% extra risk); BMR = benchmark response; HED = human equivalent dose; NA = not applicable; p-OSF = provisional oral slope factor.

The $BMDL_{10}$ (HED) of 23 mg/kg-day for the combined incidence of thyroid and Zymbal gland tumors was used as the point of departure (POD) for calculating the screening p-OSF as it was the lowest POD, compared with either of the individual tumor types. Because the study (Bucher et al., 1990; NTP, 1989) was conducted for the full lifetime of the rats (2 years), no adjustment for less-than-lifetime observation was necessary. The mode of action (MOA) by which PETN might induce Zymbal or thyroid gland tumors is not known; in the absence of definitive information, a linear approach was used to obtain the slope from the POD. The screening p-OSF of 4.3×10^{-3} $(mg/kg\text{-}day)^{-1}$ was derived as follows:

Screening p-OSF	=	BMR ÷ $BMDL_{10}$ (HED)
	=	0.1 ÷ 23 mg/kg-day
	=	**4.3×10^{-3} $(mg/kg\text{-}day)^{-1}$**

APPENDIX B. DATA TABLES

Table B-1. Acute Clinical Studies Evaluating Subjective Complaints Following PETN Administration

Study/Type	Subjects	Duration and Dose[a]	Results
Fife et al. (1958) Double-blind clinical trial, crossover design	Trial 1: 75 angina patients; 53 M/2 F; Trial 2: 42 angina patients, subset of Trial 1, sex not reported. Each subject received both placebo and PETN treatment (2 wk on, 2 wk off, with half receiving treatment each 2-wk period).	2 wk; Trial 1: 0, 2.6 mg/kg-d; Trial 2: 0, 1.3−2.6 mg/kg-d PETN, dose was 1.3 mg/kg-d for first week and 2.6 mg/kg-d for second week of the second trial, if tolerated.	"Mild" side effects (headache, giddiness, palpitation, insomnia, GI symptoms): Trial 1: 14/75 PETN, 12/75 placebo; $p > 0.1$;[b] Trial 2: 7/42 PETN, 6/42 placebo; $p > 0.1$ "Moderate to severe" side effects (headache/nausea): Trial 1: 8/75 PETN, 2/75 placebo; $p = 0.1$; Trial 2: 5/42 PETN, 0/42 placebo; $p = 0.06$
Roberts (1958) Double-blind clinical trial, crossover design	42 angina patients, 40 M/2 F. Both standard PETN and time-release were used. Each subject received a placebo and PETN treatment during the study period at 2-wk intervals; neither doctor nor patient knew what treatment was supplied at bimonthly visits.	2-wk intervals over experimental period (up to 9 mo.); 0, 0.86, 1.7 mg/kg-d	Transient effects were reported in some subjects and disappeared after a few days; effects included headache or flushing (6/42), itching (2/42), and exacerbation of existing eczema (2/42). Incidence of side effects was not reported for the placebo period.
Amsterdam et al. (1980) Double-blind clinical trial, crossover design	12 (10 M/2 F) heart failure patients 8 of the subjects received both placebo and PETN treatment; time between treatment administration was determined by "return to baseline" hemodynamics.	Once; 0, 0.57 mg/kg	Qualitative: Side effects were evaluated but not reported.
Predel et al. (1995) Double-blind clinical trial	5 healthy and 5 coronary artery disease (20 M/0 F) patients per group.	3 d; 0, 4.3 mg/kg-d	Qualitative: No side effects reported.

[a]Doses in mg/kg-day were calculated using reported body-weight means (if available) or a reference human body weight of 70 kg (U.S. EPA, 2011c).
[b]All statistics in this table were performed for this review (two-tailed Fisher's exact test).

F = female(s); GI = gastrointestinal; M = male(s); PETN = pentaerythritol tetranitrate.

Table B-2. Body Weight for F344/N Rats and B6C3F1 Mice Exposed to PETN in the Diet for 14 Days[a]

Parameter[b]	Exposure, ppm PETN (mg/kg-d)[c]					
Male rats	**0**	**620 (65.7)**	**1,240 (129.0)**	**2,500 (347.8)**	**5,000 (674)**	**10,000 (1,110)**
Terminal body weight (g)	208 ± 7	207 ± 5 (−0)	213 ± 5 (+2)	213 ± 7 (+2)	209 ± 5 (+0)	209 ± 7 (+0)
Body-weight gain (g)	69 ± 8	74 ± 2 (+7)	80 ± 1 (+16)	81 ± 4 (+17)	77 ± 2 (+12)	77 ± 3 (+12)
Female rats	**0**	**620 (79.0)**	**1,240 (168.0)**	**2,500 (355.7)**	**5,000 (635)**	**10,000 (1,310)**
Terminal body weight (g)	145 ± 3	144 ± 2 (−1)	145 ± 2 (0)	147 ± 1 (+1)	145 ± 2 (0)	138 ± 1 (−5)
Body-weight gain (g)	41 ± 2	37 ± 1 (−10)	39 ± 2 (−5)	41 ± 1 (0)	38 ± 1 (−7)	31 ± 1[d] (−24)
Male mice	**0**	**620 (173)**	**1,240 (308.7)**	**2,500 (539.7)**	**5,000 (1,380)**	**10,000 (2,600)**
Terminal body weight (g)	25.6 ± 1	25.1 ± 1.1 (−2)	25.6 ± 0.8 (0)	26.1 ± 1 (+2)	27.1 ± 1 (+6)	25.9 ± 0.8 (+1)
Body-weight gain (g)	2.7 ± 0.3	2.2 ± 0.4 (−19)	3 ± 0.3 (+11)	3.1 ± 0.5 (+15)	4.3 ± 0.3 (+59)	3.3 ± 0.2 (+22)
Female mice	**0**	**620 (187)**	**1,240 (556.9)**	**2,500 (703.1)**	**5,000 (1,800)**	**10,000 (2,530)**
Terminal body weight (g)	20.6 ± 0.4	20.2 ± 0.5 (−2)	20.1 ± 0.4 (−2)	20.1 ± 0.2 (−2)	19.7 ± 0.4 (−4)	18 ± 0.9[d] (−13)
Body-weight gain (g)	2.6 ± 0.1	2 ± 0.3 (−23)	1.9 ± 0.2 (−27)	1.8 ± 0.4 (−31)	1.1 ± 0.4[d] (−58)	0.4 ± 0.7[d] (−85)

[a]Bucher et al. (1990); NTP (1989).
[b]Data reported as mean ± SEM (% change compared with control) for 5 mice; % change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Daily doses in mg/kg-day were calculated for this review based on reported body-weight and food-consumption data.
[d]Significantly different from control ($p < 0.05$), as calculated for this review (Student's *t*-test).

PETN = pentaerythritol tetranitrate; SEM = standard error of the mean.

Table B-3. Body Weight and Relative Organ Weights in F344/N Rats Exposed to PETN in the Diet for 14 Weeks[a]

Parameter[b]	Exposure, ppm PETN (mg/kg-d)[c]					
Male	**0**	**620 (39.1)**	**1,240 (88.04)**	**2,500 (190)**	**5,000 (330)**	**10,000 (630)**
Terminal body weight (g)	339 ± 9	331 ± 8 (−2)	335 ± 7 (−1)	351 ± 6 (+4)	336 ± 8 (−1)	336 ± 6 (−1)
Body-weight gain (g)	156 ± 6	147 ± 10 (−6)	152 ± 6 (−3)	168 ± 6 (+8)	153 ± 5 (−2)	153 ± 5 (−2)
Brain (mg/g BW)	5.2 ± 0.15 [*n* = 6]	5.5 ± 0.11 (+6) [*n* = 6]	5.5 ± 0.12 (+6) [*n* = 6]	5.1 ± 0.49 (−2) [*n* = 6]	5.4 ± 0.53 (+4) [*n* = 6]	5.6 ± 0.12 (+8) [*n* = 6]
Liver (mg/g BW)	37 ± 0.88	30.6 ± 0.98** (−17)	32.5 ± 1.24* (−12)	32.9 ± 1.61 (−11)	33.1 ± 0.9 (−11)	32.7 ± 1.52 (−12)
Right kidney (mg/g BW)	4.6 ± 0.55	4.5 ± 0.6 (−2)	4.4 ± 0.54 (−4)	4.6 ± 0.61 (0)	4.5 ± 0.52 (−2)	4.6 ± 0.55 (0)
Thymus (mg/g BW)	1 ± 0.16	1 ± 0.29 (0)	0.8 ± 0.1 (−20)	1 ± 0.16 (0)	0.7 ± 0.02 (−30)	0.8 ± 0.12 (−20)
Heart (mg/g BW)	2.7 ± 0.06	2.7 ± 0.08 (0)	2.7 ± 0.08 (0)	2.9 ± 0.25 (+7)	2.7 ± 0.08 (0)	2.6 ± 0.07 (−4)
Lung (mg/g BW)	4.1 ± 0.12 [*n* = 5]	3.7 ± 0.1 (−10) [*n* = 6]	3.9 ± 0.2 (−5) [*n* = 6]	3.8 ± 0.18 (−7) [*n* = 6]	3.7 ± 0.1 (−10) [*n* = 5]	3.9 ± 0.09 (−5) [*n* = 6]
Female	**0**	**620 (42.8)**	**1,240 (85.56)**	**2,500 (200)**	**5,000 (370)**	**10,000 (830)**
Terminal body weight (g)	215 ± 2	210 ± 3 (−2)	211 ± 3 (−2)	206 ± 4 (−4)	203 ± 4[d] (−6)	201 ± 4[d] (−7)
Body-weight gain (g)	76 ± 3	70 ± 2 (−8)	72 ± 1 (−5)	67 ± 2[d] (−12)	63 ± 2[d] (−17)	62 ± 2[d] (−18)
Brain (mg/g BW)	8.3 ± 0.09	8.6 ± 0.15 (+4)	8.7 ± 0.12 (+5)	8.8 ± 0.13* (+6)	9 ± 0.13** (+8)	9 ± 0.19** (+8)
Liver (mg/g BW)	32.9 ± 0.41	32.5 ± 0.63 (−1)	31.1 ± 0.7 (−5)	32.3 ± 0.74 (−2)	31.9 ± 0.85 (−3)	33.1 ± 0.78 (+1)
Right kidney (mg/g BW)	3.1 ± 0.05	3.1 ± 0.05 (0)	3.1 ± 0.05 (0)	3.2 ± 0.06 (+3)	3.2 ± 0.05 (+3)	3.3 ± 0.06* (+6)
Thymus (mg/g BW)	1.1 ± 0.1	1 ± 0.03 (−9)	1.1 ± 0.02 (0)	1 ± 0.04 (−9)	1 ± 0.04 (−9)	0.9 ± 0.05 (−18)
Heart (mg/g BW)	2.8 ± 0.04	2.8 ± 0.05 (0)	2.8 ± 0.08 (0)	2.9 ± 0.06 (+4)	2.9 ± 0.07 (+4)	3 ± 0.06 (+7)
Lung (mg/g BW)	4.8 ± 0.11	4.6 ± 0.08 (−4)	4.7 ± 0.1 (−2)	4.8 ± 0.08 (0)	4.8 ± 0.11 (0)	4.7 ± 0.12 (−2)

[a]Bucher et al. (1990); NTP (1989).
[b]Data reported as mean ± SEM (percent change compared with control) for 10 rats, unless otherwise noted; percent change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Daily doses in mg/kg-day were calculated for this review based on daily food intake (in g/kg BW) reported by the study authors.
[d]Statistically significantly different from control ($p < 0.05$), as calculated for this review (Student's *t*-test).
*Statistically significantly different from control ($p < 0.05$), as reported by the study authors.
**Statistically significantly different from control ($p < 0.01$), as reported by the study authors.

BW = body weight; PETN = pentaerythritol tetranitrate; SEM = standard error of the mean.

Table B-4. Body Weight and Relative Organ Weights in B6C3F1 Mice Exposed to PETN in the Diet for 13 Weeks[a]

Parameter[b]	Exposure, ppm PETN (mg/kg-d)[c]					
Male	**0**	**620 (109)**	**1,240 (302.6)**	**2,500 (362.5)**	**5,000 (925)**	**10,000 (2,140)**
Terminal body weight (g)	30.9 ± 0.3	32 ± 0.6 (+4)	30 ± 0.8 (−3)	32.7 ± 0.8 (+6)	31.6 ± 0.6 (+2)	31.1 ± 0.6 (+1)
Brain (mg/g BW)	14.6 ± 0.34	14.5 ± 0.56 (−1)	14.3 ± 0.4 (−2)	14.1 ± 0.37 (−3)	14.8 ± 0.34 (+1)	15.2 ± 0.34 (+4)
Liver (mg/g BW)	52.9 ± 1.01	55.1 ± 0.97 (+4)	56.3 ± 1.13 (+6)	53.7 ± 1.49 (+2)	55.4 ± 2.13 (+5)	52.3 ± 1.34 (−1)
Right kidney (mg/g BW)	8.8 ± 0.27	8.9 ± 0.25 (+1)	8.8 ± 0.38 (0)	8.7 ± 0.21 (−1)	8.4 ± 0.16 (−5)	9.1 ± 0.21 (+3)
Thymus (mg/g BW)	1.3 ± 0.13	1.2 ± 0.11 (−8)	1.3 ± 0.26 (0)	1.2 ± 0.17 (−8)	1.2 ± 0.11 (−8)	1.3 ± 0.24 (0)
Heart (mg/g BW)	4.8 ± 0.15	4.8 ± 0.11 (0) [*n* = 9]	4.8 ± 0.14 (0)	4.5 ± 0.12 (−6)	4.7 ± 0.15 (−2)	4.8 ± 0.11 (0)
Lung (mg/g BW)	5.9 ± 0.19	6.2 ± 0.3 (+5)	6 ± 0.33 (+2)	6 ± 0.38 (+2)	6.1 ± 0.23 (+3)	6.3 ± 0.25 (+7)
Female	**0**	**620 (172)**	**1,240 (306.3)**	**2,500 (632.5)**	**5,000 (1,340)**	**10,000 (3,120)**
Terminal body weight (g)	27.3 ± 0.6	29 ± 0.7 (+6)	29.1 ± 0.7 (+7)	27.4 ± 0.6 (+0.4)	28.3 ± 0.7 (+4)	27.7 ± 0.8 (+1)
Brain (mg/g BW)	17.9 ± 0.35	17.4 ± 0.5 (−3)	17.4 ± 0.41 (−3)	17.7 ± 0.46 (−1)	17.4 ± 0.46 (−3)	18 ± 0.41 (+1)
Liver (mg/g BW)	50.2 ± 0.71	51.7 ± 1.16 (+3)	49.8 ± 0.94 (−1)	51.7 ± 0.75 (+3)	52.5 ± 1.1 (+5)	53.8 ± 0.71* (+7)
Right kidney (mg/g BW)	6.4 ± 0.1	6.8 ± 0.21 (+6)	6.3 ± 0.14 (−2)	6.6 ± 0.11 (+3)	6.7 ± 0.15 (+5)	6.9 ± 0.12* (+8)
Thymus (mg/g BW)	1.8 ± 0.16	1.6 ± 0.12 (−11)	1.8 ± 0.19 (0)	1.8 ± 0.21 (0)	1.8 ± 0.15 (0)	2 ± 0.14 (+11)
Heart (mg/g BW)	4.5 ± 0.13	4.3 ± 0.1 (−4)	4.1 ± 0.1 (−9)	4.4 ± 0.17 (−2)	4.4 ± 0.15 (−2)	4.5 ± 0.1 (0)
Lung (mg/g BW)	7.2 ± 0.27	6.9 ± 0.24 (−4)	6.3 ± 0.33 (−13)	6.5 ± 0.31 (−10)	6.6 ± 0.32 (−8)	6.8 ± 0.28 (−6)

[a]Bucher et al. (1990); NTP (1989).
[b]Data reported as mean ± SEM (percent change compared with control) for 10 mice, unless otherwise noted; percent change control = [(treatment mean − control mean) ÷ control mean] × 100.
[c]Daily doses in mg/kg-day were calculated for this review based on daily food intake (in g/kg BW) reported by the study authors.
*The *p*-value was statistically significant at < 0.05.

BW = body weight; PETN = pentaerythritol tetranitrate; SEM = standard error of the mean.

Table B-5. Incidences of Zymbal Gland and Thyroid Gland Tumors in F344/N Rats Exposed to PETN in the Diet for 2 Years[a]

		Exposure, ppm PETN (mg/kg-d)[b]		
Male		**0**	**5,000 (240)**	**10,000 (490)**
Zymbal gland	Hyperplasia	0/49 (0%)	1/45 (2%)	0/41 (0%)
	Adenoma	0/49 (0%)	1/45 (2%)	0/41 (0%)
	Carcinoma	0/49 (0%)	2/45 (4%)	2/41 (5%)
	Adenoma or carcinoma[c]	0/49 (0%)	3/45 (7%)	2/41 (5%)
	Logistic regression tests[d]	$p = 0.135$	$p = 0.108$	$p = 0.219$
	Cochran-Armitage test[d]	$p = 0.157$	NA	NA
	Fisher's exact test[d]	NA	$p = 0.106$	$p = 0.205$
Female		**0**	**1,240 (80)**	**2,500 (165)**
Zymbal gland	Hyperplasia	1/36 (3%)	0/37 (0%)	0/35 (0%)
	Adenoma	0/36 (0%)	0/37 (0%)	2/35 (6%)
	Carcinoma	0/36 (0%)	1/37 (3%)	1/35 (3%)
	Adenoma or carcinoma[e]	0/36 (0%)	1/37 (3%)	3/35 (9%)
	Logistic regression tests[d]	$p = 0.055$	$p = 0.492$	$p = 0.116$
	Cochran-Armitage test[d]	$p = 0.028$	NA	NA
	Fisher's exact test[d]	NA	$p = 0.507$	$p = 0.115$
Thyroid gland	Hyperplasia	1/50 (2%)	0/48 (0%)	1/50 (2%)
	Follicular cell adenoma or carcinoma[f]	0/50 (0%)	0/48 (0%)	3/50 (6%)
	Logistic regression trend test[d]	$p = 0.033$	NR	$p = 0.110$
	Cochran-Armitage trend test[d]	$p = 0.016$	NA	NA
	Fisher's exact test[d]	NA	NR	$p = 0.121$

[a]Bucher et al. (1990); NTP (1989).
[b]Daily doses in mg/kg-day were calculated by Bucher et al. (1990).
[c]Historical incidence at the study laboratory (mean ± SD): 4/599 (0.7 ± 1.0%); historical incidence in NTP studies: 19/1,936 (1.0 ± 1.7%, range 0−8%).
[d]The *p*-values in the control incidence are for the trend test for combined tumor incidence. The *p*-values in the exposure group incidence columns are for pairwise comparisons between that dose group and the controls for combined incidence of adenoma or carcinoma. The logistic regression test regards tumors in animals dying before terminal kill as nonfatal. The Cochran-Armitage and Fisher's exact tests directly compare the overall incidence rates.
[e]Historical incidence at the study laboratory (mean ± SD): 1/649 (0.2 ± 0.6%); historical incidence in NTP studies: 11/1,983 (0.6 ± 1.3%, range 0−6%).
[f]Historical incidence at the study laboratory (mean ± SD): 5/627 (0.8 ± 1.3%); historical incidence in NTP studies: 19/1,938 (1.0 ± 1.2%, range 0−4%).

NA = not applicable; NR = not reported; NTP = National Toxicology Program; PETN = pentaerythritol tetranitrate; SD = standard deviation.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence is as follows. The Multistage-Cancer model in the U.S. EPA's Benchmark Dose Software (BMDS, Version 2.6) is fit to the incidence data using the extra risk option. The Multistage-Cancer model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). An adequate model fit is judged by three criteria: (1) goodness-of-fit p-value ($p > 0.05$), (2) visual inspection of the dose-response curve, and (3) scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all of the models providing adequate fit to the data, the benchmark dose lower confidence limit/benchmark concentration lower confidence limit (BMDL/BMCL) for the model with the lowest Akaike's information criterion (AIC) is selected as the point of departure (POD). In accordance with U.S. EPA (2012a) guidance, benchmark dose/benchmark concentration (BMD/BMC) and BMDL/BMCL values associated with an extra risk of 10% are calculated. A combined tumor analysis using the MS-Combo model is run for each tumor type individually and then combined. A combined tumor analysis is appropriate when tumors of different clonal origin are indicated.

BMD MODELING OF CANCER ENDPOINTS

The incidence data for Zymbal gland tumors and thyroid gland tumors in female rats were modeled separately and those results are shown in Tables C-1 and C-2 and Figures C-1 and C-2. The incidence data for Zymbal gland adenoma or carcinoma in female rats exposed to dietary pentaerythritol tetranitrate (PETN) for 2 years were combined with thyroid gland adenoma or carcinoma (Bucher et al., 1990; NTP, 1989) and used for BMD modeling using an MS-Combo model.

Increased Incidence of Zymbal Gland Adenoma or Carcinoma in Female Rats Exposed to PETN for 2 Years

The procedure outlined above was applied to the data for increased combined incidence of Zymbal gland adenoma or carcinoma in female rats exposed to dietary PETN for 2 years (Bucher et al., 1990; NTP, 1989) (see Table C-1). Table C-2 summarizes the BMD modeling results. All models provided adequate fit to the data, so the model with the lowest AIC was selected (Multistage-Cancer 1st-degree). Thus, the $BMDL_{10}$ (human equivalent dose [HED]) of 27 mg/kg-day from this model is selected for this endpoint (see Figure C-1).

Increased Incidence of Thyroid Gland Adenoma or Carcinoma in Female Rats Exposed to PETN for 2 Years

The procedure outlined above was applied to the data for increased incidence of thyroid gland adenoma or carcinoma in female rats exposed to dietary PETN for 2 years (Bucher et al., 1990; NTP, 1989) (see Table C-1). Table C-2 summarizes the BMD modeling results. All models provided adequate fit to the data, so the model with the lowest AIC was selected (Multistage-Cancer 2nd-degree). Thus, the $BMDL_{10}$ (HED) of 40 mg/kg-day from this model is selected for this endpoint (see Figure C-2).

Table C-1. Combined Incidence of Zymbal Gland and Thyroid Gland Tumors in Female F344/N Rats Administered Dietary PETN for 2 Years[a]

	HED, mg/kg-d[b]		
	0	**20**	**41.3**
Sample size[c]	36/50	37/48	35/50
Zymbal gland incidence	0	1	3
Thyroid gland incidence	0	0	3

[a]Bucher et al. (1990); NTP (1989).
[b]Estimated daily animal doses were converted into HEDs based on the animal:human $BW^{1/4}$ ratio (U.S. EPA, 2005) using study-specific TWA BWs for rats and 70 kg for humans (U.S. EPA, 2011c).
[c]Note that sample sizes in the Zymbal gland and thyroid gland tumor analyses are different (see Table A-1).

BW = body weight; HED = human equivalent dose; PETN = pentaerythritol tetranitrate; TWA = time-weighted average.

Model Predictions for Zymbal Gland and Thyroid Gland Adenomas or Carcinomas in Female Rats Administered Dietary PETN for 2 Years

The procedure outlined above was applied to the data for incidence of Zymbal gland and thyroid gland adenomas or carcinomas in female rats (see Table C-1). The software converged on the Multistage-Cancer 1st-degree model for the Zymbal gland tumors and the Multistage-Cancer 2nd-degree model for thyroid tumors, which provided adequate fit ($p > 0.05$); thus, these were selected as the best-fitting models (see Table C-2). The $BMDL_{10}$ (HED) values from these models are 27.0 and 39.7 mg/kg-day, respectively. Figures C-1 and C-2 show the model fit to the data. The MS-Combo model yields a $BMDL_{10}$ (HED) value of 23 mg/kg-day, and the model output for the combined tumor analysis is shown in Table C-2.

Table C-2. BMD Model Results for Zymbal and Thyroid Gland Adenoma and Carcinoma in Female Rats Administered Dietary PETN for 2 Years[a]

Model	*df*	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residual: Dose Nearest BMD[c]	Scaled Residual: Control BMD[c]	AIC	BMD_{10} mg/kg-d	$BMDL_{10}$ mg/kg-d
Zymbal Gland Multistage-Cancer (1st-degree)[d]	**2**	**0.9224**	**0.236**	**0**	**31.84**	**55.6481**	**26.9849**
Zymbal Gland Multistage-Cancer (2nd-degree)	1	1	0	0	33.67	45.3659	27.5424
Thyroid Gland Multistage-Cancer (1st-degree)	2	0.4859	0.679	0	27.0466	104.048	45.8064
Thyroid Gland Multistage-Cancer (2nd-degree)[d]	**2**	**0.7050**	**0.361**	**0**	**25.9494**	**59.8238**	**39.6929**
Multitumor (MS-Combo)[d]	**NA**	**NA**	**NA**	**NA**	**NA**	**35.76216**	**22.59085**

[a]Bucher et al. (1990); NTP (1989).
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near BMD.
[d]Selected model. All models provided adequate fit to the data. BMDLs for models providing adequate fit were nearly identical, so the model with the lowest AIC was selected.

AIC = Akaike's information criterion; BMD = benchmark dose; BMD_{10} = 10% benchmark dose; BMDL = benchmark dose lower confidence limit (subscripts denote BMR: i.e., 10 = exposure concentration associated with 10% extra risk); *df* = degree(s) of freedom; NA = not applicable; PETN = pentaerythritol tetranitrate.

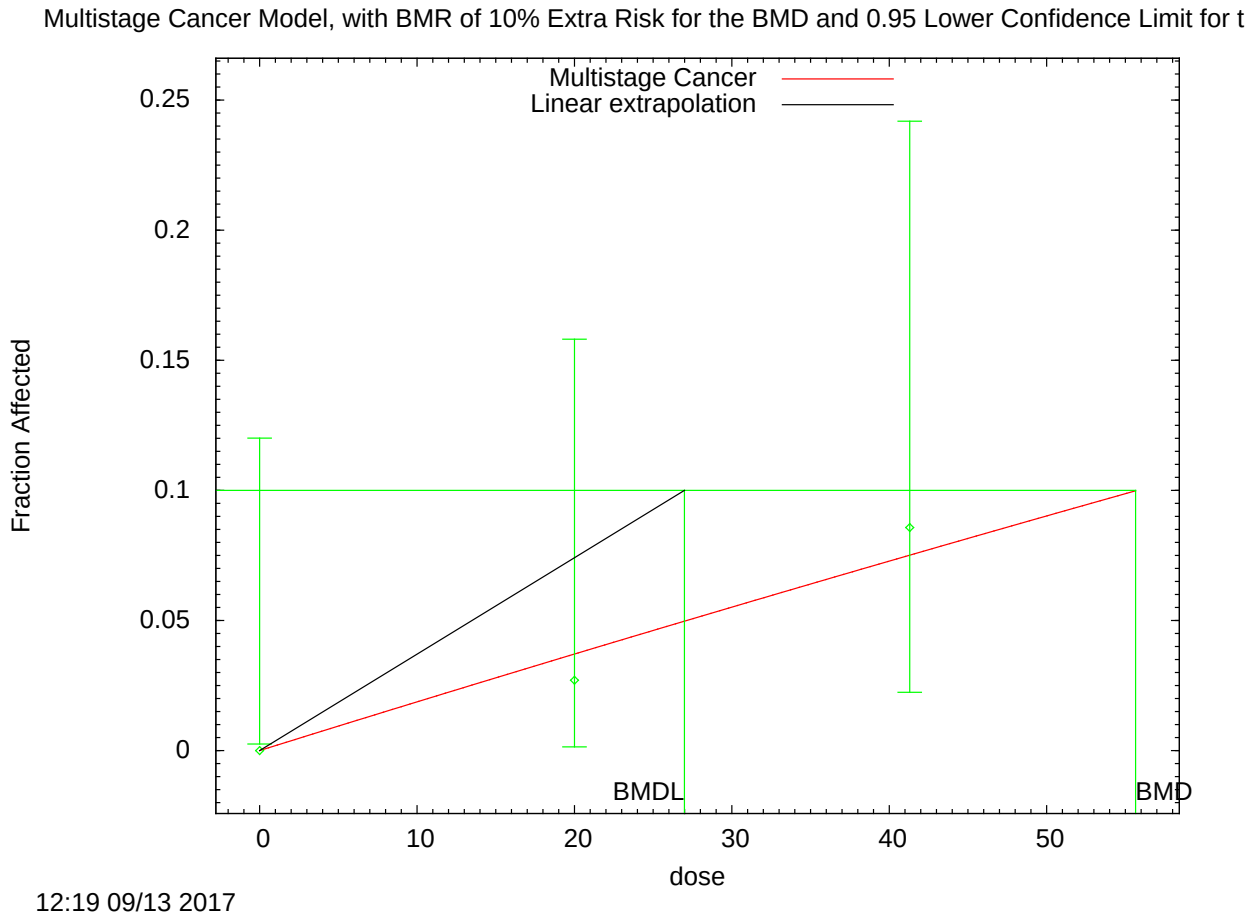

Figure C-1. Multistage (1-Degree) Model for Incidence of Zymbal Gland Adenoma or Carcinoma in Female F344/N Rats Administered Dietary PETN for 2 Years (Bucher et al., 1990; NTP, 1989)

Text Output for Figure C-1:

```
=================================================================
        Multistage Cancer Model. (Version: 1.10;  Date: 02/28/2013)
        Input Data File:
C:/Users/JKaiser/Desktop/BMDS240/Data/msc_zym_petn_Msc1-BMR10.(d)
        Gnuplot Plotting File:
C:/Users/JKaiser/Desktop/BMDS240/Data/msc_zym_petn_Msc1-BMR10.plt
                                                Wed Sep 13 12:19:57 2017
 =================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
```

```
  Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1


Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



                 Default Initial Parameter Values
                    Background =           0
                       Beta(1) =   0.00217791


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Background
                have been estimated at a boundary point, or have been specified by
the user,
                and do not appear in the correlation matrix )

               Beta(1)

   Beta(1)           1



                               Parameter Estimates

                                                      95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                   *
        Beta(1)       0.00189334           *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -14.8351         3
   Fitted model        -14.9202         1      0.170198      2          0.9184
  Reduced model        -17.1083         1       4.54653      2           0.103

          AIC:         31.8404


                               Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          36        0.000
   20.0000     0.0372         1.375     1.000          37       -0.326
   41.3000     0.0752         2.633     3.000          35        0.236

 Chi^2 = 0.16      d.f. = 2        P-value = 0.9224
```

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        55.6481

            BMDL =        26.9849

            BMDU =        205.925

Taken together, (26.9849, 205.925) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00370577
```

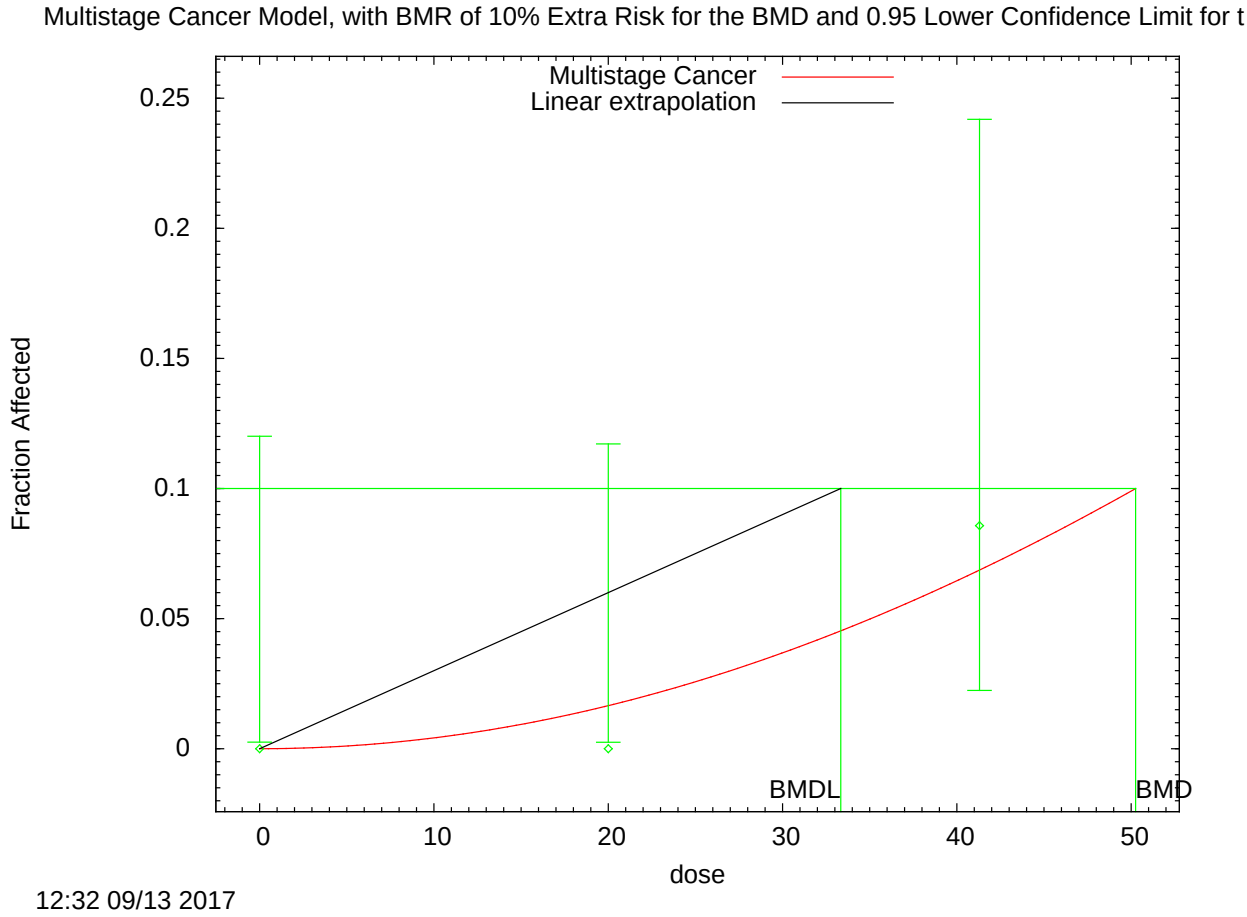

Figure C-2. Multistage (2-Degree) Model for Incidence of Thyroid Gland Adenoma or Carcinoma in Female F344/N Rats Administered Dietary PETN for 2 Years (Bucher et al., 1990; NTP, 1989)

Text Output for Figure C-2:

```
 ================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: //AA.AD.EPA.GOV/ORD/CIN/USERS/MAIN/A-E/DPETERSE/Net
MyDocuments/BMDS/BMDS2704/msc_PETN Thyroid Female_Opt.(d)
        Gnuplot Plotting File:  //AA.AD.EPA.GOV/ORD/CIN/USERS/MAIN/A-E/DPETERSE/Net
MyDocuments/BMDS/BMDS2704/msc_PETN Thyroid Female_Opt.plt
                                              Wed Sep 04 15:32:35 2019
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) = 3.90286e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)           1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0              NA
        Beta(1)                0              NA
```

```
       Beta(2)     2.94395e-005     1.69987e-005       -3.87732e-006        6.27563e-
005

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                      Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -11.3484         3
   Fitted model        -11.9747         1       1.25261      2          0.5346
  Reduced model        -14.6652         1       6.63362      2         0.03627

           AIC:         25.9494


                                 Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      50.000        0.000
   20.0000     0.0117         0.562     0.000      48.000       -0.754
   41.3000     0.0490         2.449     3.000      50.000        0.361

 Chi^2 = 0.70      d.f. = 2        P-value = 0.7050


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        59.8238

             BMDL =        39.6929

             BMDU =        243.165

Taken together, (39.6929, 243.165) is a 90     % two-sided confidence
interval for the BMD

Cancer Slope Factor =    0.00251934
```

Text Output for MS-Combo Model for Combined Incidence of Zymbal Gland Adenoma or Carcinoma and Thyroid Gland Adenoma or Carcinoma in Female F344/N Rats Administered Dietary PETN for 2 Years (Bucher et al., 1990; NTP, 1989):

```
================================================================
        MS_COMBO. (Version: 1.10;  Date: 01/29/2017)
        Input Data File: C:\Users\dpeterse\OneDrive - Environmental
Protection Agency (EPA)\Profile\Desktop\test.(d)
        Gnuplot Plotting File:  C:\Users\dpeterse\OneDrive - Environmental
Protection Agency (EPA)\Profile\Desktop\test.plt
                                        Wed Sep 04 17:17:51 2019
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = PETNZybmalFemale.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =   0.00217791


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background
                 have been estimated at a boundary point, or have been
specified by the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)           1
```

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00189334	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-14.8351	3			
Fitted model	-14.9202	1	0.170198	2	0.9184
Reduced model	-17.1083	1	4.54653	2	0.103

AIC: 31.8404

Log-likelihood Constant 12.397374590988454

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	36.000	0.000
20.0000	0.0372	1.375	1.000	37.000	-0.326
41.3000	0.0752	2.633	3.000	35.000	0.236

Chi^2 = 0.16 d.f. = 2 P-value = 0.9224

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 55.6481

BMDL = 26.9849

```
            BMDU =        205.925

Taken together, (26.9849, 205.925) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00370577


 ======================================================================
       MS_COMBO. (Version: 1.10;  Date: 01/29/2017)
       Input Data File: C:\Users\dpeterse\OneDrive - Environmental
Protection Agency (EPA)\Profile\Desktop\test.(d)
       Gnuplot Plotting File:  C:\Users\dpeterse\OneDrive - Environmental
Protection Agency (EPA)\Profile\Desktop\test.plt
                                        Wed Sep 04 17:17:51 2019
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose
   Data file name = PETNThyroidFemale.dax

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) = 3.90286e-005


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been
specified by the user,
```

and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	2.94395e-005	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-11.3484	3			
Fitted model	-11.9747	1	1.25261	2	0.5346
Reduced model	-14.6652	1	6.63362	2	0.03627

AIC: 25.9494

Log-likelihood Constant 9.8832848452188156

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	50.000	0.000
20.0000	0.0117	0.562	0.000	48.000	-0.754
41.3000	0.0490	2.449	3.000	50.000	0.361

Chi^2 = 0.70 d.f. = 2 P-value = 0.7050

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

```
Confidence level =          0.95

             BMD =        59.8238

            BMDL =        39.6929

            BMDU =        243.165

Taken together, (39.6929, 243.165) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00251934


**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                     -26.894855234855342

  Combined Log-likelihood Constant             22.280659436207269


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        35.7621

            BMDL =        22.5908

            BMDU =        61.8096

Multistage Cancer Slope Factor =    0.00442657
```

APPENDIX D. REFERENCES

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