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Provisional Peer-Reviewed Toxicity Values for

Picric Acid (2,4,6-Trinitrophenol) (CASRN 88-89-1)

and

Ammonium Picrate (CASRN 131-74-8)



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

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

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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 PICRIC ACID (CASRN 88-89-1) AND AMMONIUM PICRATE (CASRN 131-74-8)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations.

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

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

QUALITY ASSURANCE

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

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

DISCLAIMERS

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

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

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

QUESTIONS REGARDING PPRTVS

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

INTRODUCTION

Picric acid, CASRN 88-89-1, also known as 2,4,6-trinitrophenol, is a pale yellow, odorless crystalline solid used in the manufacture of explosives, batteries, matches, and dyes for textiles (O'Neil et al., 2013). The chemical formula of picric acid is $C_6H_3N_3O_7$ and its chemical structure is presented in Figure 1.

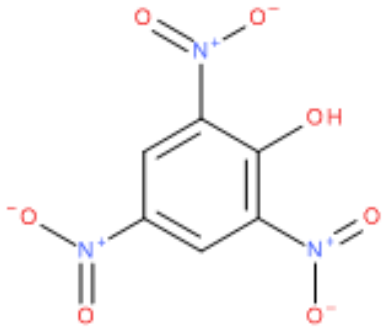


Figure 1. Picric Acid (CASRN 88-89-1) Structure

Ammonium picrate, CASRN 131-74-8, is the ammonium salt of picric acid. Ammonium picrate occurs as bright yellow scales or orthorhombic crystals (O'Neil et al., 2013). It is used in explosives, fireworks, and rocket propellants (O'Neil et al., 2013). The empirical formula for ammonium picrate is $C_6H_6N_4O_7$ (see Figure 2).

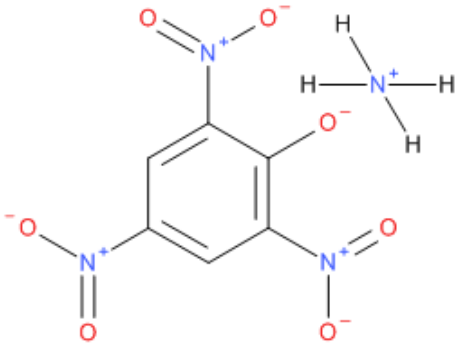


Figure 2. Ammonium Picrate (CASRN 131-74-8) Structure

A table of physicochemical properties for picric acid and ammonium picrate is provided below (see Table 1). Both picric acid and ammonium picrate are water soluble. The low pKa of 0.38 for picric acid corresponds to a high pKb of 13.62 for its conjugate base, the picrate anion. The high pKb indicates that in aqueous solution at neutral pH, the picrate ion will be mostly dissociated from any spectator cation that is present (e.g., hydrogen ion for picric acid, ammonium ion for ammonium picrate). As a result, both picric acid and ammonium picrate

rapidly dissociate to form the picrate anion when dissolved in water (Thorne and Jenkins, 1997). Even in the acid environment of the stomach (pH = 3−4 in rats, pH = 1.5−3.5 in humans), both ammonium picrate and picric acid will occur primarily as dissociated picrate anions, and will do so as well in other parts of the body with more neutral pH levels. Therefore, ammonium picrate is expected to behave like picric acid in the body, and the systemic toxicities of the two chemicals are expected to be very similar. Although available absorption, distribution, metabolism, and excretion (ADME) studies are limited, they have shown the picrate anion to be present in the blood and urine of rabbits after dosing (intraperitoneal [i.p.] or dermal) with both picric acid and ammonium picrate (Weeks et al., 1983). Distribution of the picrate anion was widespread in rabbits and guinea pigs following inhalation exposure to ammonium picrate. Additionally, although toxicity data for ammonium picrate are limited, lethal doses by i.p. injection in rats were similar for both compounds (Weeks et al., 1983). Further support for the similarities of ammonium picrate and picric acid is provided in the analogue approach presented in Appendix A. In the review that follows, data from both picric acid and ammonium picrate exposures are considered together in developing assessments of picrate anion toxicity that are applicable to both picrate source compounds.

Table 1. Physicochemical Properties of Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Property (unit)	Picric Acid Value	Ammonium Picrate Value
Boiling point (°C)	300 (explodes)[a]	423 (explodes)[a]
Melting point (°C)	122.5[b]	Decomposes[a]
Density (g/mL at 20°C)	1.76[a]	1.72[a]
Vapor pressure (mm Hg at 25°C)	7.5×10^{-7} [b]	3.4×10^{-11} (estimated)[b]
Henry's law constant (atm-m^3/mole at 25°C)	1.70×10^{-11} (estimated)[b]	2.9×10^{-22} (estimated)[b]
pH (unitless)	0.2 (colorless) to 1 (yellow)[c]	NV
Solubility in water (mg/L at 25°C)	1.27×10^{4} [b]	1.6×10^{5} (estimated)[b]
Relative vapor density (air = 1)	7.9[a]	NV
Log K_{ow}	1.33[b]	−1.40 (estimated)[b]
pKa (at 25°C)	0.38[b]	NA
pKb	NA	13.62[d]
Molecular weight (g/mol)	229.1[b]	246.1[b]

[a]Lewis (2012).
[b]ChemIDplus (2018).
[c]ChemicalBook (2017).
[d]Calculated using the equation pKb = pKw – pKa (conjugate acid) = 14 − 0.38 = 13.62, where Kw is the ionic equilibrium constant for water (10^{-14}).

NA = not applicable; NV = not available.

A summary of available toxicity values for picric acid and ammonium picrate from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference[c]
Noncancer			
IRIS	NV	NA	U.S. EPA (2018b)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2018a)
ATSDR	NV	NA	ATSDR (2019)
IPCS	NV	NA	IPCS (2018)
CalEPA	NV	NA	CalEPA (2018a); CalEPA (2018b)
OSHA (PEL-TWA)	0.1 mg/m^3 (picric acid)	Skin designation; 8-hr TWA for general industry, construction, and shipyard employment	OSHA (2018a); OSHA (2018b); OSHA (2020)
NIOSH (REL-TWA)	0.1 mg/m^3 (picric acid)	Skin designation; TWA for up to a 10-hr workday	NIOSH (2016)
NIOSH (STEL-TWA)	0.3 mg/m^3 (picric acid)	15-min TWA exposure that should not be exceeded at any time during a workday	NIOSH (2016)
NIOSH (IDLH)	75 mg/m^3 (picric acid)	Based on acute oral toxicity data in humans and animals	NIOSH (1994)
ACGIH (TLV-TWA)	0.1 mg/m^3 (picric acid)	Based on skin sensitization, dermatitis, and eye irritation	ACGIH (2001); ACGIH (2018)
DOE (PAC)	PAC-1: 0.3 mg/m^3 (picric acid) PAC-2: 17 mg/m^3 PAC-3: 100 mg/m^3	Based on TEELs	DOE (2016)
DOE (PAC)	PAC-1: 30 mg/m^3 (ammonium picrate) PAC-2: 330 mg/m^3 PAC-3: 2,000 mg/m^3	Based on TEELs	DOE (2016)
USAPHC (air-MEG)	1-hr critical: 75 mg/m^3 (picric acid) 1-hr marginal: 15 mg/m^3 1-hr negligible: 0.30 mg/m^3 8-hr negligible: 0.10 mg/m^3 14-d negligible: 0.034 mg/m^3 1-yr negligible: 0.034 mg/m^3	1-hr values based on TEELs; other values based on TLV for skin sensitization, dermatitis, and eye irritation	U.S. APHC (2013)
USAPHC (air-MEG)	1-hr critical: 250 mg/m^3 (ammonium picrate) 1-hr marginal: 50 mg/m^3 1-hr negligible: 30 mg/m^3	Based on TEELs	U.S. APHC (2013)
Cancer			
IRIS	NV	NA	U.S. EPA (2018b)
HEAST	NV	NA	U.S. EPA (2011a)
DWSHA	NV	NA	U.S. EPA (2018a)

Table 2. Summary of Available Toxicity Values for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Source (parameter)[a, b]	Value (applicability)	Notes	Reference[c]
CalEPA	NV	NA	CalEPA (2018a); CalEPA (2018b)
NTP	NV	NA	NTP (2016)
IARC	NV	NA	IARC (2018)
ACGIH	NV	NA	ACGIH (2018)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DOE = Department of Energy; 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; USAPHC = U.S. Army Public Health Command.
[b]Parameters: IDLH = immediately dangerous to life or health; MEG = military exposure guideline; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; STEL = short-term exposure limit; TEEL = temporary emergency exposure limit; TLV = threshold limit value; TWA = time-weighted average.
[c]Reference date is the publication date for the database and not the date the source was accessed.

NA = not applicable; NV = not available.

A non-date-limited literature search was last updated in April 2020 for picric acid (CASRN 88-89-1), and non-date-limited searches were conducted in April 2020 for studies relevant to the derivation of provisional toxicity values for ammonium picrate (CASRN 131-74-8). The database searches for PubMed, TOXLINE (including TSCATS1), and Web of Science were conducted by an information specialist and records stored in the U.S. EPA's Health and Environmental Research Online (HERO) database. The following additional databases were searched for health-related data: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), 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 Health Effects Assessment Summary Tables (HEAST), U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), Japan Existing Chemical Data Base (JECDB), National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Organisation for Economic Co-operation and Development (OECD) Existing Chemicals Database, OECD Screening Information Dataset (SIDS) High Production Volume Chemicals via International Programme on Chemical Safety (IPCS) INCHEM, Occupational Safety and Health Administration (OSHA), U.S. Army Public Health Command (APHC), and World Health Organization (WHO).

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide an overview of the relevant data for picric acid and ammonium picrate and include all potentially relevant repeated-dose, short-term, subchronic, and chronic studies. Principal studies used in the PPRTV assessment for derivation of provisional toxicity values are identified in bold. The phrase "statistical significance" or the term "significant," used throughout the document, indicates a p-value < 0.05 unless otherwise noted.

Table 3A. Summary of Potentially Relevant Noncancer Data for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[b]	Critical Effects	NOAEL	LOAEL	Reference (comments)	Notes[c]
Human							
1. Oral (mg/kg-d)							
ND							
2. Inhalation (mg/m^3)							
ND							
Animal							
1. Oral (mg/kg-d)							
Short term	4 M/4 F, S-D rat, picric acid administered by gavage, newborn rat dose-finding study, daily for 14 d (PND 4−17) Reported doses: 0, 16.3, 81.4, or 407 mg/kg-d	0, 16.3, 81.4, or 407	Decreased body weight in males and females, decreased relative and absolute kidney weight in males, and increased relative liver weight in males and females. Mortality was observed in males and females.	16.3	81.4 (FEL)	Takahashi et al. (2004)	PR
Short term	6 M/6 F, S-D rat, picric acid administered by gavage, newborn rat main study, daily for 18 d (PND 4−21) Reported doses: 0, 4.1, 16.3, or 65.1 mg/kg-d	0, 4.1, 16.3, or 65.1	Increased relative and absolute liver weight in males and females, decreased absolute epididymis weight in males and increased relative spleen weight in females.	16.3	65.1	Takahashi et al. (2004)	PR
Short term	3 M/3 F, S-D rat, picric acid administered by gavage, young rat dose-finding study daily for 14 d Reported doses: 0, 20, 100, or 500 mg/kg-d	0, 20, 100, or 500	Hematological effects in females, increased liver weight in males and females, increased relative spleen weight in males.	20	100	Takahashi et al. (2004)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[b]	Critical Effects	NOAEL	LOAEL	Reference (comments)	Notes[c]
Short term	**6 M/6 F, S-D rat, picric acid administered by gavage, young rat main study, daily for 28 d** **Reported doses: 0, 4, 20, or 100 mg/kg-d**	**0, 4, 20, or 100**	**Increased absolute liver weights, hematological and related splenic effects (increased absolute and relative spleen weights and hematopoiesis) in males and females, and testicular effects (testicular atrophy, decreased sperm in the epididymis) in males**	**20**	**100**	**Takahashi et al. (2004)**	**PR, PS**
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) for oral noncancer effects and as TWA concentrations (mg/m^3) for inhalation noncancer effects.
[c]Notes: PS = principal study; PR = peer reviewed.

ADD = adjusted daily dose; F = females; FEL = frank effect level; LOAEL = lowest-observed-adverse-effect level; M = males; ND = no data; NOAEL = no-observed-adverse-effect level; PND = postnatal day; S-D = Sprague-Dawley; TWA = time-weighted average.

Table 3B. Summary of Potentially Relevant Cancer Data for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Category	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry	Critical Effects	Reference (comments)	Notes
Human					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					
Animal					
1. Oral (mg/kg-d)					
ND					
2. Inhalation (mg/m^3)					
ND					

ND = no data.

HUMAN STUDIES

Oral Exposures

ACGIH (2001) reports that ingestion of 1−2 g picric acid (approximately 14−28 mg/kg) can cause severe poisoning in humans. Effects attributed to picric acid poisoning include headache, vertigo, nausea, vomiting, abdominal cramps, diarrhea, yellow coloration of the skin and conjunctiva, myalgia, hematuria, albuminuria, and at high doses, destruction of erythrocytes, gastroenteritis, hemorrhagic nephritis, acute hepatitis, stupor, convulsions, and death (ACGIH, 2018; NIOSH, 2016; ILO, 2011; Weeks et al., 1983). No data were located on the toxicity of ammonium picrate to humans following repeated oral exposure.

Inhalation Exposures

Exposure to picric acid dust in the air has been reported to produce skin and eye irritation and sensitization in exposed persons (ACGIH, 2001). No data on systemic or respiratory effects of inhaled picric acid were found. One published study evaluated potential effects of occupational inhalation exposure to ammonium picrate dust for 2−24 months (Sunderman et al., 1945).

Sunderman et al. (1945)

Sunderman et al. (1945) evaluated the potential health effects in 71 individuals who worked predominately with ammonium picrate for 2−24 months. Other chemicals that the workers may have been exposed to included potassium nitrate and chlorinated diphenyl. The employees worked in 10 different types of positions with varying exposure to ammonium picrate dust. Those working in "milling" and "preforming" positions (n = 18) were exposed to the most dust, with atmospheric concentrations at these sites ranging from 0.0088 to 0.1942 mg/m^3, based on 20-minute samples collected every 2 hours over several working days. Respirators from two milling workers collected 52 and 156 mg of ammonium picrate during a 6-hour operation. Atmospheric dust concentrations were not reported for other working areas (pressing, examining, coating, firing, pack house, laundry, or experimental). Physical examinations were conducted over a 1−15-month period in all workers, including urinalysis, complete blood counts, and skin evaluation. Upon report of epistaxis (acute hemorrhage from the nostril, nasal cavity, or nasopharynx) by two workers, examination of the nasal cavity, pharynx, ear canal, drum membrane, epipharynx, larynx, hearing ability with 4,090 double vibrations (d.v.) frequency tuning fork, and inspection and palpation of the neck for enlarged lymph nodes was conducted on 18 randomly selected workers.

Results of the urinalysis and blood testing were not reported. Physical examination of the workers found swelling and excoriation (skin lesions due to chronic skin-picking) of the nasal mucous membranes and nasal mucosa that bled at slight manipulation in several individuals. Yellowish skin coloration (particularly around the hairline, nape of the neck, and palms) and dermatitis were also observed in several workers, but this was attributed to direct skin exposure to dust rather than inhalation exposure. Dermatitis was observed in 7/71 workers on exposed parts of the body (hands/forearms). All seven workers were in "low exposure" areas (coating, firing, laundry, experimental). Lesions, characterized as erythematous patches containing papules and vesicles, cleared when individuals were removed from contact with ammonium picrate. Two of the individuals developed eczematous lesions and could not continue to work with ammonium picrate; the other five individuals were able to return to work with additional protection (i.e., gloves). In the detailed evaluation of the upper respiratory tract, yellow discoloration of the nasal vestibules and yellow stained mucus in the anterior turbinate were

observed. The investigators found that blowing ammonium picrate dust into the nose caused a slight reflexive spasm in the palatal pharyngeal area, which was associated with a "tight feeling, sharp taste, odor" described by most of the workers. No alterations in the other examined endpoints were attributable to inhalation of ammonium picrate dust.

The investigators concluded that inhalation of ammonium picrate dust does not cause prominent pathological changes in humans. However, available data are inadequate to establish no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) values because of the lack of a control group; relatively small study size; limitations in characterization of exposure and evaluation of health endpoints, and reporting deficiencies in the publication.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to picric acid were evaluated in two short-term toxicity studies and two corresponding dose-finding studies (Takahashi et al., 2004).

Short-Term Studies

Takahashi et al. (2004): Newborn Rat Dose-Finding Study

Sprague-Dawley (S-D) rat pups (4/sex/dose) were administered picric acid (81.4% purity) by daily gavage at doses of 0, 16.3, 81.4, or 407 mg (as picric acid)/kg-day from Postnatal Days (PNDs) 4−17 (14 days total). For the gavage doses, the picric acid was suspended in a 0.5% carboxymethyl cellulose sodium salt aqueous solution with 0.1% Tween-80. One foster mother nursed four male and four female pups. The animals were allowed free access to a sterilized basal diet (manufacturer [MF]: Oriental Yeast, Tokyo, Japan) and were maintained in an environmentally controlled room at 22 ± 2°C with a relative humidity of 55 ± 10% and a 12:12-hour light/dark cycle. All pups were sacrificed on PND 18 and necropsies conducted. General condition, body weight, hematology, blood biochemistry, and organ weights were examined.

In the high-dose group, all pups died by Day 4 of the dosing period. In the mid-dose group, one male pup died on Day 3, a female pup died on Day 6, and a second female pup died on Day 7 of the dosing period. Prior to their deaths, these pups showed hypoactivity, bradypnea, and hypothermia. In surviving pups in the mid-dose group, hypoactivity was observed on Days 3, 5, or 8 of the dosing period. Yellowish fur was observed in all picric acid-treated rats but not in the controls; the study authors stated that this "did not seem to be an adverse effect" because the pups' "hair roots and skin showed no anomalies." The mid-dose treatment resulted in statistically significant decreases in body weight in the male pups (13% lower than controls). Decreased body weights were also observed in the female pups (15% lower than controls), but the decreases were not statistically significant (see Table B-1). The study authors reported that no treatment-related effects were observed on food consumption or behavior. They also reported that treatment at the mid dose resulted in statistically significant increases in relative liver weights (13%, liver-to-body–weight ratio) in male pups and statistically nonsignificant increases in relative liver weights in female pups (22%; see Table B-1). The mid-dose animals also showed statistically significant decreases in absolute (26%) and relative (14%) kidney weight in male pups and statistically nonsignificant decreases in absolute (22%) and relative (8.2%) kidney weight in female pups. No other significant body-weight and organ-weight changes were reported. The study authors also stated that there were no other consistent changes related to the administration of picric acid in hematological results, blood biochemical parameters, or necropsy

findings at any dose. A frank effect level (FEL) of 81.4 mg/kg-day is identified for this study based on mortality observed in male and female rat pups; a corresponding NOAEL of 16.3 mg/kg-day is determined. Significant (statistically and/or biologically) changes in body, kidney, and liver weight in male and/or female rats were also observed at 81.4 mg/kg-day.

Takahashi et al. (2004):18-Day Newborn Rat Main Study

In a peer-reviewed, short-term, toxicity study performed by Takahashi et al. (2004), picric acid was suspended in a 0.5% carboxymethyl cellulose sodium salt aqueous solution with 0.1% Tween-80 (81.4% purity) and given to six pup S-D rats/sex/dose daily via gavage. Test sample impurities included: 18.5% (w/w) water and 0.008% (w/w) sulfuric acid (based on personal communication with the study corresponding author). The study authors reported administered doses of 0, 4.1, 16.3, or 65.1 mg (as picric acid)/kg-day to pups from PNDs 4−21 (18 days). The pups in the main study were sacrificed on PND 22. Another six pups/sex/dose in the maintenance-recovery groups were given the same dosages for 18 days, then maintained for 9 weeks without chemical treatment and sacrificed on PND 85. Twelve foster mothers were used to suckle the pups up to PND 22. The animals were allowed free access to a sterilized basal diet (MF: Oriental Yeast, Tokyo, Japan) after weaning and were maintained in an environmentally controlled room at 24 ± 2°C with a relative humidity of 55 ± 10% and a 12:12-hour light/dark cycle. The study authors reported using Good Laboratory Practice (GLP) principles.

General condition was observed twice daily for pups and foster mothers during the dosing period and daily for pups during the recovery-maintenance period. All pups were examined for developmental landmarks such as pinna detachment (PND 4), piliation (PND 8), incisor eruption (PND 10), gait and eye opening (PND 15), testis descent (PND 21), and preputial separation and/or vaginal opening (PND 42). Body weights were recorded, and food consumption was determined at least twice per week. Body weights were also measured on the day of testis descent and preputial separation and/or vaginal opening. Blood was collected from the abdominal vein on the day of sacrifice, and the following hematological parameters were evaluated: erythrocyte or red blood cell (RBC) count, hematocrit (Hct), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte or white blood cell (WBC) count, differential leukocyte count, platelet count (PLAT), mean platelet volume (MPV), cell morphology, prothrombin time (PT), and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were also examined: total protein (TP), triglycerides (TRI), albumin (A), globulin (G), albumin:globulin ratio (A:G), glucose (GLU), cholesterol (CHOL), total bilirubin (TBILI), blood urea nitrogen (BUN), creatinine (CREAT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALK), calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and chloride (Cl). After gross examination, the liver, kidney, spleen, thymus, pituitary gland, adrenals, lungs, gonads, heart, and brain were weighed. Tissue samples from these organs were also fixed, sectioned, and histologically examined.

No treatment-related effects were noted on food consumption, mortality, or behavior in the main study. Yellowish fur was observed in all picric acid-treated rats but not in the controls. The study authors reported a statistically significant decrease in body weight on Days 4 and 8 of the dosing period (maximum 7% decrease) for males in the 65.1-mg/kg-day group (data not presented in publication). However, terminal body weights for treated groups in the main study

were not statistically different from controls (see Table B-2). No dose-dependent effects on body weight or food consumption were observed during the maintenance-recovery period. As shown in Table B-2, males and females in the 65.1-mg/kg-day dose groups showed statistically significant increases (13 and 12%, respectively) in relative liver weights (liver-to-body–weight ratio) compared with controls. Absolute liver weights were also increased in males and females in the 65.1-mg/kg-day dose groups (10 and 12%, respectively), although these increases did not reach statistical significance. The males in the same dose group had statistically significant decreases in absolute (but not relative) epididymis weight, and the females had statistically significant increases in relative (but not absolute) spleen weight. No other treatment-related, organ-weight effects were observed. Developmental landmarks and sexual maturation were similar in the treated and control groups. No treatment-related changes in hematological parameters, urinalysis, clinical chemistry measurements, or histopathological findings were reported in males or females. Based on increased absolute and relative liver weights in both sexes, decreased absolute epididymis weight in males and increased relative spleen weight in females, the high dose of 65.1 mg/kg-day is considered the LOAEL and the mid dose of 16.3 mg/kg-day is identified as the corresponding NOAEL for both male and female rats.

Takahashi et al. (2004): Young Rat Dose-Finding Study

Five-week-old S-D rats (3/sex/dose) were administered picric acid (81.4% purity) by daily gavage at doses of 0, 20, 100, or 500 mg (as picric acid)/kg-day for 14 days. Picric acid was suspended in a 0.5% carboxymethyl cellulose sodium salt aqueous solution with 0.1% Tween-80. The animals were sacrificed on the day following the last dose after overnight fasting and necropsies were conducted. General condition, body weight and food consumption, hematology results, and organ weights were examined.

In the high-dose group, all male rats and one female rat died by Day 2 of the dosing period. No deaths were observed in the control, low- and mid-dose groups. Yellowish fur was observed in all picric acid-treated rats but not in controls; the study authors stated that this "did not seem to be adverse" because the rats' "hair roots and skin showed no anomalies." They also reported that the body weights of males and females in the low- and mid-dose groups did not significantly differ from those of controls during the dosing period. No treatment-related effects were reported on food consumption or behavior. Both males and females in the mid-dose group exhibited significantly increased absolute liver weights (13% increase in males and 25% in females) and relative liver weights (9.7% and not statistically significant in males and 18% in females; see Table B-3). Mid-dose males exhibited a statistically significant increase in relative spleen weight (14% increase). No other statistically significant organ-weight changes were reported. Mid-dose females exhibited significantly lower Hb and Hct values and a higher reticulocyte (Ret) ratio relative to controls (see Table B-4). A LOAEL of 100 mg/kg-day with a corresponding NOAEL of 20 mg/kg-day is identified for this study based on biologically significantly (≥10%) increased liver weights in male (absolute) and female (absolute and relative) rats and increased relative spleen weight in male rats.

Takahashi et al. (2004):28-Day Young Rat Main Study

In a separate study by Takahashi et al. (2004), picric acid was given to young (5-week-old) S-D rats (six/sex/dose) daily via gavage. This study used the same vehicle with identical measured impurities as the newborn rat study. The study authors reported administering doses of 0, 4, 20, or 100 mg (as picric acid)/kg-day to rats in the main study for 28 days. The animals were sacrificed the next day following an overnight fast. Another

six rats/sex/dose in the maintenance-recovery groups were given 0 or 100 mg/kg-day picric acid starting on Week 5 for a total of 28 days, then maintained for 2 weeks without chemical treatment and sacrificed on Week 11. The animals were allowed free access to a sterilized basal diet (MF: Oriental Yeast, Tokyo, Japan) after weaning. They were examined for general condition, body weight, organ weight, food consumption, urinalysis, hematology, blood biochemistry, necropsy, and histopathological findings as described for the newborn study.

There were no treatment-related effects on mortality, food consumption, or body weight during the dosing or maintenance-recovery periods. Yellowish fur was observed in all picric acid-treated rats but not in the controls. As shown in Table B-5, there were statistically significantly higher WBC and Ret counts and lower RBC and Hb levels in males at 100 mg/kg-day. In females exposed to the highest dose, there were statistically significant increases in WBC, Ret, MCV, and lower RBC, Hb, and MCHC.

Statistically significant changes in relative liver weight (12% increased), absolute spleen weight (44% increased), relative spleen weight (45% increased), absolute epididymis weight (23% decreased), and relative epididymis weight (23% decreased) were observed at the end of the dosing period in males at 100 mg/kg-day only (see Table B-6). The only statistically significant changes at the end of the maintenance-recovery period were in absolute epididymis weight (25% decreased) and relative epididymis weight (17% decreased) in males at the 100 mg/kg-day dose. In females, there were statistically significant increases in relative liver weight (23%), absolute spleen weight (92%), and relative spleen weight (100%) at the end of the 28-day dosing period at the highest dose only (see Table B-6). No statistically significant changes in organ weight were observed in females at the end of the maintenance-recovery period. Although statistically significant changes in absolute liver weight were not observed in exposed male and female rats, biologically significant (>10%) increases occurred in the high-dose group for both sexes. No other organ-weight changes were reported. Statistically significant histopathological changes occurred in males at the highest dose at the end of the dosing period and included development of germinal centers and extramedullary hematopoiesis in the spleen, testicular atrophy, and decreased sperm in the epididymis (see Table B-7). Females at 100 mg/kg-day showed the development of germinal centers, extramedullary hematopoiesis, and hemosiderin deposition in the spleen at the end of the dosing period (see Table B-8). At the end of the maintenance-recovery period, only hemosiderin deposition in the spleen of both males and females and testicular atrophy in males were observed at 100 mg/kg-day (data not shown). No other changes were reported. Based on hematological and related splenic effects, increased liver weights and testicular effects, the high dose of 100 mg/kg-day is identified as the LOAEL and the mid dose of 20 mg/kg-day is the corresponding NOAEL.

Subchronic Studies

No studies have been identified.

Chronic Studies

No studies have been identified.

Reproductive Studies

No studies have been identified.

Developmental Studies

No studies have been identified.

Inhalation Exposures

No adequate inhalation studies have been identified on the subchronic, chronic, developmental, or reproductive toxicity or on the carcinogenicity of picric acid or ammonium picrate in animals.

OTHER DATA

Table 4A provides an overview of genotoxicity studies of picric acid and ammonium picrate, and Table 4B provides and overview of other supporting studies on picric acid and ammonium picrate.

Table 4A. Summary of Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8) Genotoxicity						
Endpoint	**Test System**	**Dose/ Concentration[a]**	**Results without Activation[b]**	**Results with Activation[b]**	**Comments**	**References**
Genotoxicity studies in prokaryotic organisms						
Mutagenicity (picric acid)	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537	0−100 µg picric acid/plate	(−) TA98, TA100, TA1535, TA1537	(−) TA1535 (±) TA100 (+) TA98, TA1537	Activation using male S-D rat liver S9 induced with Aroclor 1254	Haworth et al. (1983)
Mutagenicity (picric acid)	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537	0−100 µg picric acid/plate	(−) TA98, TA100, TA1535, TA1537	(−) TA1535, TA100 (+) TA98, TA1537	Activation using male Syrian hamster liver S9 induced with Aroclor 1254	Haworth et al. (1983)
Mutagenicity (ammonium picrate)	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	0, 0.5, 1.0, 10, 100, 500, 1,000 µg ammonium picrate/plate	−	−	Plate incorporation	Litton Bionetics (1979)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutagenicity (ammonium picrate)	*Saccharomyces cerevisiae* strain D4	0, 0.5, 1.0, 10, 100, 500, 1,000 µg ammonium picrate/plate	−	−	Plate incorporation	Litton Bionetics (1979)

Table 4A. Summary of Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8) Genotoxicity						
Endpoint	**Test System**	**Dose/ Concentration[a]**	**Results without Activation[b]**	**Results with Activation[b]**	**Comments**	**References**
Genotoxicity studies in mammalian cells—in vitro						
Mutagenicity (ammonium picrate)	L5178Y/TK+/− mouse lymphoma cells	0, 500−3,000 µg ammonium picrate/mL (−S9) 0, 31.3−1,000 µg ammonium picrate/mL (+S9)	−	−	Cytotoxicity (>50%) was observed at ≥500 µg/mL without activation and ≥400 µg/mL with activation. In the assay with activation, only 220 cells were scored at 1,000 µg/mL due to high toxicity. Precipitation was noted at concentrations >1,000 µg/mL; these concentrations were obtained by mixing the weighed compound directly into growth medium.	Litton Bionetics (1979)
Clastogenicity [CA] (picric acid)	CHO cells	0, 600, 800, 1,000 µg picric acid/mL (−S9) 0, 1,740, 2,485, 3,500, 5,000 µg picric acid/mL (+S9)	–	−	NA	NTP (1985)
Clastogenicity [SCE] (picric acid)	CHO cells	0, 50, 167, 500, 1,700 µg picric acid/mL (−S9) 0, 167, 500, 1,670, 5,000 µg picric acid/mL (+S9)	+	−	NA	NTP (1985)

Table 4A. Summary of Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8) Genotoxicity						
Endpoint	**Test System**	**Dose/ Concentration[a]**	**Results without Activation[b]**	**Results with Activation[b]**	**Comments**	**References**
Clastogenicity [SCE] (ammonium picrate)	L5178Y/TK+/− mouse lymphoma cells	0, 15.6, 31.3, 62.5, 125, 250 µg ammonium picrate/mL (−S9); 0, 1.00, 2.00, 3.90, 7.80, 15.6, 31.3 µg ammonium picrate/mL (+S9)	+	−	Significantly increased SCE frequency was observed at concentrations ≥15.6 µg/mL (compared with solvent control) and at 250 µg/mL (compared with negative control). Cytotoxicity was observed at concentrations >31.3 µg/mL in the presence of S9 activation: cultures at these concentrations did not contain scoreable cells.	Litton Bionetics (1979)
Genotoxicity studies—in vivo						
Mutagenicity [dominant lethal] (ammonium picrate)	Male CD-1 mice (10/group); ammonium picrate administered in deionized water orally once/d for 5 d and mated weekly to untreated virgin females over 7 wk; females were sacrificed after 14 d	0, 2.23, 7.43, 22.3 mg ammonium picrate/kg	−		No significant difference from concurrent and/or historical controls for the parameters measured.	Litton Bionetics (1979)

Table 4A. Summary of Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8) Genotoxicity

Endpoint	Test System	Dose/ Concentration[a]	Results without Activation[b]	Results with Activation[b]	Comments	References
Mutagenicity [sex-linked recessive lethal] (picric acid)	*Drosophila melanogaster*	0, 450 ppm picric acid (feeding); 0, 400 ppm picric acid (injection)	– (feeding; injection)		Data represent results from three different laboratories. All laboratories obtained negative results from feeding studies; however, exposure after injection yielded positive results in one laboratory. The study authors also noted that when all experimental data are combined, the findings compared to controls are significant (p = 0.02).	Woodruff et al. (1985)
		0, 300, 500, 1,000, 1,500 ppm picric acid (feeding); 0, 1,000, 1,500 ppm picric acid (injection)	– (feeding; injection)			
		0, 1,250 ppm picric acid (feeding); 0, 1,500 ppm picric acid (injection)	– (feeding) + (injection)			
Clastogenicity [mouse bone marrow CA] (ammonium picrate)	Male Ha/ICR mice (32/group); ammonium picrate administered in deionized water orally for 1 (single dose) or 5 d; sacrifice at 6, 24, or 48 hr following single exposure; or 6 hr following final exposure of 5 d.	0, 2.23, 7.43, or 22.3 mg ammonium picrate/kg	–		NA	Litton Bionetics (1979)
Clastogenicity [reciprocal translocation] (picric acid)	*D. melanogaster*	0, 1,500 ppm picric acid (injection)	–		NA	Woodruff et al. (1985)

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = positive, (+) = weak positive, – = negative, ± = equivocal.

CA = chromosomal aberration; CHO = Chinese hamster ovary; NA = not applicable; S-D = Sprague-Dawley; SCE = sister chromatid exchange.

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Supporting evidence—human studies				
Dermal [patch test] (ammonium picrate)	Patch-testing of 23 volunteers, (11 picrate workers, 12 never-exposed individuals). Patches of acetone saturated with ammonium picrate were placed on the inside of the arm for 5 d. Ten days after removal of the patch, the patch was reapplied for 2 d. Skin was evaluated for irritation.	No skin irritation was observed in any subject after the initial exposure. Two individuals reacted after reapplication of patches to the same area (one picrate worker and one nonpicrate worker). However, the nonpicrate worker was a guard who had previously reported itchy, burning, reddened eyelids upon making rounds in the building containing ammonium picrate.	Ammonium picrate is not a primary irritant but may cause sensitization in some individuals.	Sunderman et al. (1945)
Dermal [case study] (picric acid)	A 61-yr-old woman presented with severe acute eczema on dorsum of right hand after using Queratil® burn cream for 3 d. The cream contained 10% alcoholic solution of picric acid along with several other compounds. After the burn healed and eczema cleared, patch testing was conducted.	Patch testing was positive for a reaction to picric acid. Patch testing was negative to other cream components.	Observed allergic reaction was due to sensitization to picric acid.	Aguirre et al. (1993)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Supporting evidence—animal studies				
Acute [oral] (picric acid)	The LD_{50} was determined in groups of rats (at least 3/sex/group) exposed to picric acid at doses of 0, 50, 100, 200, 600, 400, or 800 mg/kg via gavage. Rats were observed for 14 d. Separate groups of males (2−3/group) were exposed to 0, 100, 200, 300, or 400 mg/kg. Arterial blood acid-base parameters were measured 35−40 min after exposure.	Mortality in males was 0% at ≤200 mg/kg, 70–80% at 300−400 mg/kg, and 100% at 800 mg/kg. In females, death was 0% at ≤100 mg/kg, ~25% at 200 mg/kg, and 100% at ≥300 mg/kg. Death occurred within 60 min at lethal doses. Clinical signs at lethal doses included tremors leading to tonic/clonic convulsions and discharge from the eyes. Cause of death was attributed to acidosis, with significant fall in blood pH at 400 mg/kg.	Rat LD_{50}: Male: 290 ± 57.5 mg/kg Female: 200 ± 42.9 mg/kg	Wyman et al. (1992)
Acute [oral] (ammonium picrate; picric acid)	The LD_{50} was determined in groups of male and female rats exposed to ammonium picrate or picric acid in polyethylene glycol via gavage. Rats were observed for 14 d.	Clinical signs at lethal doses (compound not specified) included ataxia, convulsions, and red discharge from eyes.	Rat LD_{50} (CI = 95%): Ammonium picrate Male: 1,690 (1,590−1,830) mg/kg Female: 720 (240−2,120) mg/kg Picric acid Male: 629 (536−737) mg/kg Female: 520 (430−620) mg/kg	Weeks et al. (1983)
Acute [oral] (picric acid)	The LD_{LO} for oral picric acid was determined in groups of cats and rabbits. No further details were provided.	NA	Cat LD_{LO} = 250 mg/kg Rabbit LD_{LO} = 120 mg/kg	Weeks et al. (1983)
Acute [i.p.] (ammonium picrate; picric acid)	The ALD was determined in groups of male and female rats exposed to ammonium picrate or picric acid in polyethylene glycol via i.p. injection. Rats were observed for 14 d.	Clinical signs at lethal doses (compound not specified) included ataxia, convulsions, and red discharge from eyes.	Rat ALD: Ammonium picrate Male: 168 mg/kg Female: 168 mg/kg Picric acid Male: 378 mg/kg Female: 168 mg/kg	Weeks et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Chronic [inhalation] (ammonium picrate)	Four rabbits and 8 guinea pigs were housed in cages in the milling and preforming areas of a factory with reported ammonium picrate dust concentrations of 0.0088−0.1942 mg/m^3. No control animals were included. Sacrifices were scheduled at 6 wk (2 rabbits) and 12 mo (remaining animals). At death or sacrifice, "tissues" were examined for histopathological changes.	Three guinea pigs died during exposure (Wk 1, Wk 3, and 9 mo). Histopathological examination of the guinea pig that died after 3 wk showed congestion, inflammation, and erosion of the nasal mucosa and turbinates, lung congestion, and hyaline degeneration of the heart. Inflammation of the trachea and periductal fibrosis and glycogen infiltration of the liver was observed in rabbits sacrificed at 6 wk. After 12 mo, histopathological findings included minor inflammatory lesions in the lungs and yellow picrate deposits in several organs, most notably the lungs and liver but also the heart, thyroid, and kidney.	Very few conclusions can be drawn from this study because of the lack of controlled exposure, limited exposure level data, and lack of a control group. However, the study suggests that ammonium picrate may damage the respiratory tract.	Sunderman et al. (1945)
Acute [dermal] (ammonium picrate; picric acid)	Six rabbits were exposed once to 0.5 g ammonium picrate or picric acid under occlusion on intact or abraded skin. The skin was evaluated 24 hr, 72 hr, and 7 d after a single application.	No skin irritation was observed.	Neither ammonium picrate nor picric acid are primary skin irritants.	Weeks et al. (1983)
Acute [dermal] (ammonium picrate)	Guinea pigs (10/group) were injected with 0 or 1% solution of ammonium picrate in polyethylene glycol for 3 wk. After a 2-wk rest, guinea pigs were challenged with single challenge dose.	2/10 guinea pigs showed sensitization reactions (compared with 0/10 controls).	Ammonium picrate is a mild skin sensitizer.	Weeks et al. (1983)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
Supporting evidence—studies of ADME				
ADME [oral] (picric acid)	Blood and urine samples were collected from F344 rats treated via gavage with a single dose of [^{14}C] picric acid (100 mg/kg).	[^{14}C]-label absorption from the gut increased continuously for 1 hr, then was eliminated in a biphasic manner. A large majority remained in the gut (absorption coefficient was 0.069 hr^{-1}). 24 hr after exposure, the primary depots of radioactivity (per gram tissue basis) were blood (plasma protein binding), spleen, kidney, liver, lung, and testes. 50.55% of radioactivity was found in the urine, 22.75% was in the gut contents, and 6.19% was in feces. The following metabolites were isolated from urine: *N*-acetylisopicramic acid (14.8%), picramic acid (18.5%), *N*-acetylpicramic acid (4.7%), and unidentified components (2.4%). Most of the radioactivity was in the parent compound (60%) that was excreted unchanged.	Absorption from the gut is limited. Absorption and elimination are biphasic with an initial rapid phase. The slow phase is attributed to plasma protein binding. Distribution is widespread. Primary metabolic pathways are reduction of the ortho or para nitro group on the aromatic ring and acetylation of the amine. Primary urinary excretion product is the parent compound (which exists as dissociated picrate anion under physiological conditions).	Wyman et al. (1992)

Table 4B. Other Studies				
Test	**Materials and Methods**	**Results**	**Conclusions**	**References**
ADME [i.v.] (picric acid)	Blood and urine samples were collected from F344 rats following i.v. injection of [^{14}C] picric acid (50 mg/kg).	Blood distribution period of 2 hr. Elimination followed first-order kinetics, with a plasma half-life of 13.4 hr (rapid phase) followed by a slower elimination phase attributed to plasma protein binding (demonstrated in vitro). 24-hr post-injection, 81.5% of i.v. dose was cleared from the blood, with 58.9% excreted in urine and 12.2% eliminated in feces. Low levels still found in urine and feces 14 d after i.v. dose.	Elimination is biphasic, with initial (rapid) half-life of 13.4 hr. Low levels are retained in the body for long periods of time (attributed to plasma protein binding). The primary excretion path is urinary.	Wyman et al. (1992)
ADME [i.p.] (ammonium picrate; picric acid)	Blood and urine samples were collected from rabbits following i.p. injection of ammonium picrate or picric acid (60 mg/kg).	Ammonium picrate: Blood concentrations of picrate ion were 135.7, 87.8, 12.1, and 0 µg/mL at 1 hr, 6 hr, 24 hr, and 7 d, respectively. Urine concentrations of picrate ion were 2.6, 0.7, 0, and 0 µg/mL at 24 hr, 48 hr, 72 hr, and 7 d, respectively. Picric acid: Blood concentrations of picrate ion were 93.5, 7.7, and 0 µg/mL at 6 hr, 24 hr, and 7 d, respectively. Urine concentrations of picrate ion were 4.7, 1.5, 1.2, and 0 µg/mL at 24 hr, 48 hr, 72 hr, and 7 d, respectively.	Data suggest biphasic elimination, with peak blood concentrations at ≤24 hr, but detectable picrate ion in urine at 3 d.	Weeks et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
ADME [dermal] (ammonium picrate; picric acid)	Blood and urine samples were collected from rabbits following dermal exposure to ammonium picrate or picric acid for 24 hr under occluded conditions (600 mg/kg).	Ammonium picrate: Blood concentrations of picrate ion were 8.8, 31.3, and 7.9 µg/mL at 6 hr, 24 hr, and 7 d, respectively. Urine concentrations of picrate ion were 11.6, 14.4, 21.8, and 2 µg/mL at 24 hr, 48 hr, 72 hr, and 7 d, respectively. Picric acid: Blood concentrations of picrate ion were 84.7, 69.7, and 0 µg/mL at 6 hr, 24 hr, and 7 d, respectively. Urine concentrations of picrate ion were 112.5, 18.0, 10.3, and 1.3 µg/mL at 24 hr, 48 hr, 72 hr, and 7 d, respectively.	Data suggest biphasic elimination, with peak blood concentrations at ≤24 hr, but detectable picrate ion in urine at 7 d.	Weeks et al. (1983)
ADME [inhalation] (ammonium picrate)	Tissue samples were examined for picrate deposits in rabbits and guinea pigs housed in cages in the milling and preforming areas of a factory for 12 mo. Reported ammonium picrate dust concentrations of 0.0088−0.1942 mg/m^3.	Picrate deposits as demonstrated as guanidine (as a fixing agent) picrate granules were found in various organs, with highest concentrations in lung and liver, followed by moderate concentrations in heart, thyroid, and kidneys. Small amounts were seen in the chondrocytes of the bronchial cartilages and adrenal cells.	Distribution is widespread following inhalation exposure.	Sunderman et al. (1945)

ADME = absorption, distribution, metabolism, excretion; ALD = approximate lethal dose; CI = confidence interval; i.p. = intraperitoneal; i.v. = intravenous; LD_{50} = median lethal dose (dose at which 50% mortality occurs); LD_{LO} = lowest observed lethal dose; NA = not applicable.

Genotoxicity

Evidence for genotoxic activity of picric acid and ammonium picrate is limited. Neither picric acid nor ammonium picrate was mutagenic to *Salmonella typhimurium* without metabolic activation (Haworth et al., 1983; Litton Bionetics, 1979). In the presence of metabolic activation, however, picric acid was mutagenic to *S. typhimurium* strains TA98 and TA1537 (Haworth et al., 1983), while ammonium picrate was not (Litton Bionetics, 1979). Ammonium picrate was also not mutagenic to *Saccharomyces cerevisiae* strain D4 or mouse L5178Y/TK+/− lymphoma cells with or without metabolic activation (Litton Bionetics, 1979). Results were negative in in vivo assays for dominant lethal mutations in mice treated orally with ammonium picrate (Litton Bionetics, 1979) and in sex-linked recessive lethal mutations in *Drosophila melanogaster* treated with picric acid orally or by injection (one injection study was positive, but this result was not confirmed in two additional studies) (Woodruff et al., 1985).

Studies evaluating clastogenicity found that both picric acid and ammonium picrate induced sister chromatid exchanges (SCE) in mammalian cells without, but not with, metabolic activation (NTP, 1985; Litton Bionetics, 1979). Picric acid did not increase chromosomal aberrations (CAs) in Chinese hamster ovary (CHO) cells with or without metabolic activation in vitro (NTP, 1985), and ammonium picrate was negative in an assay for CAs in mouse bone marrow cells in vivo (Litton Bionetics, 1979). Picric acid was also negative in a test for reciprocal translocations in *D. melanogaster* in vivo (Woodruff et al., 1985).

Supporting Human Studies

As already discussed, dermatitis observed in several workers exposed to ammonium picrate dust for 2−24 years was suggestive of skin sensitization (Sunderman et al., 1945). Follow-up patch testing showed no evidence of primary irritation but did show positive sensitization reactions in 2/23 volunteers (Sunderman et al., 1945). A case of allergic contact dermatitis due to picric acid in a burn cream has also been reported (Aguirre et al., 1993).

Supporting Animal Toxicity Studies

Oral LD_{50} values in rats ranged from 200−629 mg/kg for picric acid and from 720−1,690 mg/kg for ammonium picrate (Wyman et al., 1992; Weeks et al., 1983). Minimum lethal oral doses for picric acid were reported as 250 mg/kg for cats and 120 mg/kg for rabbits (Weeks et al., 1983). Approximate lethal doses in rats treated by intraperitoneal (i.p.) injection were 168 mg/kg for ammonium picrate and 168−378 mg/kg for picric acid (Weeks et al., 1983). Clinical signs at lethal doses in these studies included ataxia, tremors, convulsions, and discharge from the eyes (Wyman et al., 1992; Weeks et al., 1983).

Sunderman et al. (1945) evaluated potential effects of industrial exposure to ammonium picrate dust in four rabbits and eight guinea pigs. The animals were housed in cages in the milling and preforming areas of a factory with reported dust concentrations of 0.0088−0.1942 mg/m^3 for up to 12 months. Three guinea pigs died during exposure (Week 1, Week 3, and 9 months). Histopathological examinations of the animals that died and those sacrificed after 12 months indicate that exposure may be associated with damage to the respiratory tract. However, no conclusions can be drawn from this study because of the lack of a control group; the small group sizes; limitations in characterization of exposure and evaluation of health endpoints, and reporting deficiencies.

Neither ammonium picrate nor picric acid produced primary skin irritation in rabbits exposed topically to 0.5 g under occlusion on intact or abraded skin (Weeks et al., 1983). Ammonium picrate tested positive for dermal sensitization in 2/10 guinea pigs (Weeks et al., 1983).

Metabolism/Toxicokinetic Studies

Wyman et al. (1992) evaluated the toxicokinetics of picric acid in rats following oral or intravenous exposure. This study indicated that gastrointestinal absorption is approximately 60−80%. Distribution of the absorbed compound was widespread, with the largest disposition in the blood (due to plasma protein binding), followed by the spleen, kidney, liver, lung, and testes. Elimination is biphasic, with an initial rapid plasma half-life of 13.4 hours, followed by evidence of slower elimination (detectable levels in urine 14 days postexposure). Excretion is primarily via urine, and the majority of the excretion product (60%) is the parent compound, which exists as dissociated picrate anion under physiological conditions. Metabolites identified in the urine (*N*-acetylisopicramic acid, picramic acid, *N*-acetylpicramic acid) indicate that the primary metabolic pathways are reduction of the ortho or para nitro group on the aromatic ring and acetylation of the amine. Studies evaluating picrate deposits following dermal or i.p. exposure to picric acid or ammonium picrate support biphasic elimination, with peak blood concentrations at ≤24 hours but low levels still detectable 3−7 days after exposure (Weeks et al., 1983).

Sunderman et al. (1945) reported widespread deposition of picrate in rabbits and guinea pigs housed in cages in a factory with ammonium picrate dust concentrations of 0.0088−0.1942 mg/m^3 for up to 12 months. The highest concentrations were in the lungs and liver, with lower concentrations detected in the heart, thyroid, and kidneys.

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF ORAL REFERENCE DOSES

The database of the oral toxicity studies for picric acid includes two short-term toxicity studies in rats and two dose-finding studies in rats, all of which were conducted by Takahashi et al. (2004). The dose-finding studies were not considered for deriving the p-RfDs because the follow-up primary studies reported in the same publication were more comprehensive and because the dose-finding studies had some limitations, such as smaller sample sizes. No repeated-dose oral toxicity studies have been identified for ammonium picrate.

Derivation of a Subchronic Provisional Reference Dose

The studies by Takahashi et al. (2004) were peer reviewed and employed GLP principles. In the 18-day newborn-rat study, a NOAEL of 16.3 mg/kg-day and a LOAEL of 65.1 mg/kg-day are identified for both males and females based on increased absolute and relative liver weight in males and females, decreased absolute epididymis weight in males, and increased relative spleen weight in females. No treatment-related histopathological findings were reported in the liver or any other organ examined. In the 28-day young-rat study, a NOAEL of 20 mg/kg-day and a LOAEL of 100 mg/kg-day are identified for males and females based on splenic, hematological, testicular, and liver effects.

In U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, U.S. EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for deriving an oral reference dose (RfD) under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects. A validated human physiologically based toxicokinetic model for picric acid is not available for use in extrapolating doses from animals to humans. Furthermore, the most sensitive endpoints being considered are not portal-of-entry effects. The $BW^{3/4}$ scaling factor was not applied to effects in newborn rats because empirical data are currently lacking on whether $BW^{3/4}$ scaling is appropriate for extrapolating from neonates or juveniles across species. However, scaling by $BW^{3/4}$ is considered relevant for deriving HEDs for effects observed in the young rats that were 5 weeks old when treatment began.

Following U.S. EPA (2011b) guidance, the doses administered resulting in the most sensitive endpoints are converted to HEDs through application of a dosimetric adjustment factor (DAF) derived as follows:

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

where

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

Study-specific body weight (i.e., terminal body weight in Table B-6) is used to calculate the DAF for each dose group (U.S. EPA, 2011b). Calculated HEDs for the young-rat main experiment in the Takahashi et al. (2004) study can be found in Tables B-3 through B-6.

Benchmark dose (BMD) analyses were conducted on the liver weight, absolute epididymis weight, and relative spleen weight data from the newborn rat study using the U.S. EPA's Benchmark Dose Software (BMDS; Version 2.7). Animal doses reported by the study authors were used in the BMD modeling for effects in newborn rats as discussed above. All the data were adequately fitted with the BMD model suite and results of BMD modeling are summarized in Appendix C. The lowest benchmark dose lower confidence limit (BMDL) identified from the newborn rat study is 34 mg/kg-day ($BMDL_{10}$) based on increased absolute liver weight in females (see Table C-1).

BMD analyses were also conducted on the statistically or biologically significant blood, organ-weight, and epididymis-weight data from the young rat study. Although statistically significant, histopathological data from the young rat study are not amenable to BMD modeling because changes only occurred at the highest dose and no changes occurred at low and mid-doses. Specifically, splenic lesions only occurred in males and females at the highest treatment dose (100 mg/kg-day), and testicular lesions in males were also reported only at this dose. Before BMD modeling, all experimental doses were converted to a human equivalent dose (HED) based on animal terminal body weight reported in the study. The lowest BMDL identified from the young rat study is 1.2 mg/kg-day (HED) based on increased WBC count in males; however, the level of response for this effect to be considered biologically significant is unclear (see Table C-2). The next lowest BMDL from the young rat study is 1.8 mg/kg-day (HED) based on increased absolute spleen weight in males. Although spleen weights in male and female rats were most prominently increased at the highest dose (26.9 mg/kg-day [HED]), slight elevations also occurred at lower doses. Furthermore, trend test analyses revealed that treatment-related increments in absolute spleen weight in males were highly significant (analysis of variance [ANOVA] contrast with equally spaced coefficients; trend $p = 9.6 \times 10^{-5}$). Consistent findings of decreases in RBC and Hb levels in both male and female rats, increases in absolute and relative spleen weights, and multiple histopathological findings on the spleen suggest a treatment-induced hematological response and point to the spleen as the major target organ. Thus, the $BMDL_{1SD}$ (HED) of 1.8 mg/kg-day based on increased absolute spleen weight in males from the young rat study is selected as the point of departure (POD) for deriving the subchronic provisional reference dose (p-RfD). The POD of 1.8 mg/kg-day (HED) based on the young rat study is lower than the most sensitive POD of 34 mg/kg-day (ADD) based on increased absolute liver weight in newborn females; therefore, it will be protective for effects observed in newborn animals exposed to picric acid.

The POD (HED) based on picric acid data reflects toxicity of the picrate anion, and for reasons described in the "Introduction" section, is considered to be applicable to both picric acid and ammonium picrate, which are equivalent picrate sources in the environment and in the body.

Because the molecular weight of ammonium picrate (formula weight [FW] = 246.1) is slightly higher than picric acid (FW = 229.1), the same POD (HED) of 1.8 mg/kg-day for picric acid is used, for practical reasons, for ammonium picrate in deriving the subchronic p-RfD without formula weight adjustment.

The subchronic p-RfD for picric acid and ammonium picrate, based on the $BMDL_{1SD}$ (HED) of 1.8 mg/kg-day for increased absolute spleen weight in male rats exposed to picric acid, is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= BMDL_{1SD}\ (HED) \div UF_C \\ &= 1.8\ mg/kg\text{-}day \div 300 \\ &= \mathbf{6 \times 10^{-3}\ mg/kg\text{-}day} \end{aligned}$$

Table 5 summarizes the uncertainty factors for the subchronic p-RfD for picric acid and ammonium picrate.

Table 5. Uncertainty Factors for the Subchronic p-RfD for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for remaining uncertainty (e.g., the toxicodynamic differences between rats and humans) following oral picrate exposure. The toxicokinetic uncertainty has been accounted for by calculation of an HED through application of a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied because there are no acceptable developmental or two-generation reproductive toxicity studies, although there is limited examination of reproductive parameters in the newborn rat study. In addition, the database lacks repeated-dose oral studies beyond 28-d exposure for picric acid and repeated-dose oral studies of any duration for ammonium picrate.
UF_H	10	A UF_H of 10 is applied to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of picric acid or ammonium picrate in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a 28-d rat study was selected as the principal study.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; 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 picric acid and ammonium picrate is low, as explained in Table 6 below.

Table 6. Confidence Descriptors for the Subchronic p-RfD for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	Confidence in the key study is medium. The Takahashi et al. (2004) study had a duration of only 28 d and it used a small number of animals. However, this study is appropriate in the number of endpoints analyzed; it is peer-reviewed and the experiments were performed according to GLP principles.
Confidence in database	L	There are no acceptable developmental or two-generation reproductive toxicity studies and no repeated-dose studies beyond 28-d exposure.
Confidence in subchronic p-RfD	L	The overall confidence in the subchronic p-RfD is low.

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

GLP = Good Laboratory Practice; L = low; M = medium; p-RfD = provisional reference dose.

Derivation of a Chronic Provisional Reference Dose

There are no chronic oral studies available for picric acid or ammonium picrate. Furthermore, the longest available study is 28 days in duration, which is not suitable for deriving a chronic p-RfD because of increased uncertainty in extrapolating to a chronic-duration time frame. However, Appendix A of this document contains a screening value (screening chronic p-RfD) using an analogue (e.g., structural, metabolic, and toxicity-like) approach, which may be of use under certain circumstances. Based on the overall analogue approach presented in Appendix A, 1,3,5-trinitrobenzene was selected as the most appropriate analogue for picric acid and ammonium picrate for deriving a screening chronic p-RfD.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Human and animal data are inadequate to derive subchronic or chronic p-RfCs for picric acid and ammonium picrate. The only available repeated-exposure study is an occupational study by Sunderman et al. (1945) that reported a lack of prominent pathological changes in humans exposed to ammonium picrate dust for 2−24 months; however, this study has major limitations (including lack of adequate exposure monitoring) that preclude identification of a NOAEL or LOAEL value. Sunderman et al. (1945) also evaluated potential adverse effects in laboratory animals by housing rabbits and guinea pigs in the factory near areas of high exposure. This study suggested potential damage to the respiratory tract but was not adequate to draw any conclusions.

Table 7 summarizes noncancer oral and inhalation reference values derived.

Table 7. Summary of Noncancer Reference Values for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (HED)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Increased absolute spleen weight	6×10^{-3}	$BMDL_{1SD}$	1.8 (based on picric acid)	300	Takahashi et al. (2004)
Screening chronic p-RfD (mg/kg-d)	Rat/M	Increased MetHb and spleen-erythroid cell hyperplasia	2×10^{-3}	NOAEL	0.643 (based on the selection of 1,3,5-trinitrobenzene as the analogue)	300	Reddy et al. (2001); Reddy et al. (1997)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; M = male; MetHb = methemoglobin; NDr = not determined; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; SD = standard deviation; UF_C = composite uncertainty factor.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 8 identifies the cancer weight-of-evidence (WOE) descriptor for picric acid and ammonium picrate. No cancer data are available for either chemical. Genotoxicity assays of both picric acid and ammonium picrate (see Table 4A) have yielded mixed results. Under the U.S. EPA (2005) cancer guidelines, the available data are inadequate to assess human carcinogenic potential, and the cancer WOE descriptor for picric acid and ammonium picrate is *"Inadequate Information to Assess the Carcinogenic Potential"* (for both oral and inhalation routes of exposure).

Table 8. Cancer WOE Descriptor for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to Humans"	NS	NA	There are no human carcinogenicity data identified to support this descriptor.
"Likely to Be Carcinogenic to Humans"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"Suggestive Evidence of Carcinogenic Potential"	NS	NA	There are no animal carcinogenicity studies identified to support this descriptor.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Both**	**This descriptor is selected due to the lack of any information on carcinogenicity of picric acid or ammonium picrate.**
"Not Likely to Be Carcinogenic to Humans"	NS	NA	No evidence of noncarcinogenicity is available.

NA = not applicable; NS = not selected; WOE = weight of evidence.

DERIVATION OF PROVISIONAL CANCER RISK ESTIMATES

Due to lack of carcinogenicity data for picric acid and ammonium picrate, derivation of cancer risk estimates is precluded (see Table 9).

Table 9. Summary of Cancer Risk Estimates for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

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

NDr = not determined; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. SCREENING PROVISIONAL VALUES

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

APPLICATION OF AN ALTERNATIVE ANALOGUE APPROACH

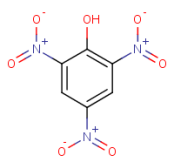
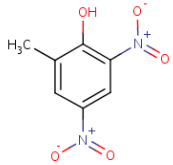
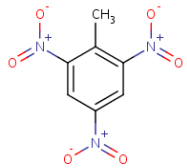
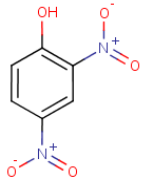
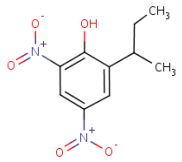
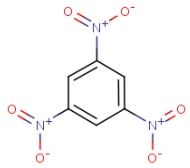
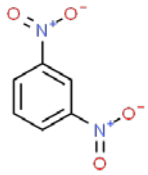
The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012). Three types of potential analogues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

In this document, the analogue approach has been applied with the goal of deriving a chronic p-RfD. No adequate data were available to support derivation of inhalation toxicity values via the analogue approach. Furthermore, this document has been developed for picric acid and ammonium picrate, which are both ready (water soluble and rapidly dissociated to form picrate anion), and equivalent sources of picrate anion in the environment and in the body. In application of the analogue approach below, only picric acid was explicitly considered, because available metabolic and repeated-dose toxicological data were only available for this chemical. Nevertheless, the results are considered applicable to both picrate source compounds.

Structural Analogues

An initial analogue search focused on identifying structurally similar chemicals with toxicity values from the Integrated Risk Information System (IRIS), PPRTV, and Health Effects Assessment Summary Tables (HEAST) databases to take advantage of the well-characterized chemical-class information. The search was accomplished through U.S. EPA's DSSTox database (DSSTox, 2012) at similarity levels >60% and the National Library of Medicine's (NLM's) ChemIDplus database (ChemIDplus, 2018) at similarity levels >80%. Six structural analogues to picric acid were identified to have oral toxicity values listed on IRIS or a PPRTV: 2-methyl-4,6-dinitrophenol (U.S. EPA, 2010); 2,4,6-trinitrotoluene (U.S. EPA, 1989b); 2,4-dinitrophenol (U.S. EPA, 1991); 2-(1-methylpropyl)-4,6-dinitrophenol (U.S. EPA, 1989a); 1,3,5-trinitrobenzene (U.S. EPA, 1997); and 1,3-dinitrobenzene (U.S. EPA, 1988a). Table A-1 summarizes their physicochemical properties and similarity scores.

Table A-1. Physicochemical Properties of Picric Acid (CASRN 88-89-1) and Candidate Structural Analogues

Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitrotoluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitrobenzene	1,3-Dinitrobenzene
Structure							
CASRN	88-89-1	534-52-1	118-96-7	51-28-5	88-85-7	99-35-4	99-65-0
Molecular weight[a]	229.10	198.133	227.132	184.11	240.214	213.105	168.108
DSSTox similarity score (%)	100	78	58.3	99	60.6	73.6	73.6
ChemIDplus similarity score (%)[a]	100	83.86	83.51	80.26	80.17	75.03	57.25
Melting point (°C)[a]	122.5	86.6	80.1	115.5	40	121.5	90
Boiling point (°C)[a]	300[b]	378	NV	NV	332	315	291
Vapor pressure (mm Hg [at °C])[a]	7.50×10^{-7} (at 25°C)	1.06×10^{-4} (at 25°C)	8.02×10^{-6} (at 25°C)	3.90×10^{-4} (at 20°C)	NV	NV	NV
Henry's law constant (atm-m^3/mole [at °C])[a]	1.70×10^{-11} (at 25°C)	1.4×10^{-6} (at 25°C)	2.08×10^{-8} (at 25°C)	8.60×10^{-8} (at 20°C)	4.56×10^{-7} (at 25°C)	3.31×10^{-10}	4.90×10^{-8}
Water solubility (mg/L [at °C])[a]	1.27×10^{4} (at 25°C)	198 (at 20°C)	130 (at 25°C)	2,790 (at 25°C)	52 (at 25°C)	278 (at 15°C)	533 (at 25°C)
Log K_{ow}[a]	1.33	2.12	1.6	1.67	3.56	1.18	1.49
pKa[a]	0.38 (at 25°C)	4.31 (at 21°C)	NV	4.09 (at 25°C)	4.62	NV	NV

[a]ChemIDplus (2018).
[b]ChemicalBook (2017).

NV = not available.

Metabolic Analogues

Table A-2 summarizes the available toxicokinetic data for picric acid and the structurally similar compounds identified as candidate analogues. Experimental data indicate that picric acid and all six potential analogues are absorbed following oral exposure, have widespread distribution, and are primarily eliminated via the urine. While no identical metabolites have been identified between picric acid and candidate analogues, nitro reduction is a common metabolic pathway for all compounds (see Table A-2). Common subsequent or secondary pathways included acetylation (picric acid, 2-methyl-4,6-dinitrophenol, 2,4,6-trinitrotoluene, 1,3-dintrobenzene), conjugation with sulfate or glucuronic acid (Dinoseb, 1,3-dintrobenzene), ring hydroxylation (2,4,6-trinitrotoluene, 1,3-dintrobenzene), and oxidation of methyl group (2-methyl-4,6-dinitrophenol, 2,4,6-trinitrotoluene, Dinoseb). Taken together, available data indicate similar patterns of absorption, distribution, and excretion, as well as a common primary metabolic pathway (nitro reduction) for picric acid and candidate analogues. Therefore, all candidate analogues are considered potential metabolic analogues for picric acid.

Table A-2. Comparison of Available ADME Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

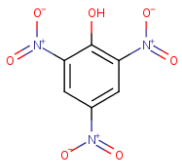
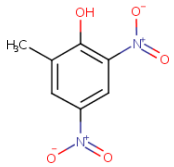
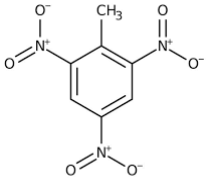
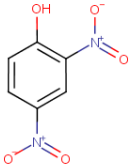
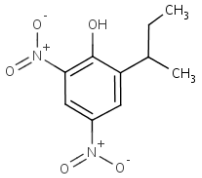
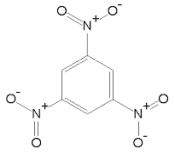
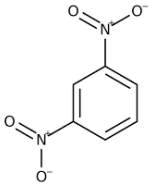
2,4,6-Trinitrophenol (Picric acid) CASRN 88-89-1	**2-Methyl-4,6-dinitrophenol (DNOC) CASRN 534-52-1**	**2,4,6-Trinitrotoluene (TNT) CASRN 118-96-7**	**2,4-Dinitrophenol (2,4-DNP) CASRN 51-28-5**	**2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb) CASRN 88-85-7**	**1,3,5-Trinitrobenzene CASRN 99-35-4**	**1,3-Dinitrobenzene CASRN 99-65-0**
						
Absorption						
Oral absorption in rats was ~60−80% based on radioactivity in blood, urine, and tissues.	Oral absorption in humans was ~40%, based on radioactivity in urine and blood. Oral absorption in rats was ~60%, based on radioactivity in the blood, urine, and tissues.	Oral absorption in rats, mice, and dogs was ≥60%, based on radioactivity in urine.	Oral absorption is rapid in mice; extent of absorption was not reported.	Oral absorption in rats and mice was ≥80% based on elimination via urine and feces (see below).	Oral absorption in rats was ≥24−39% based on urine and expired air (see below); fecal elimination was low, and it is uncertain whether the balance of the dose was retained in the body or if overall recovery was low.	Oral absorption in rabbits was ≥80% based on elimination via urine (see below).
Distribution						
<u>Rats exposed orally:</u> Widespread, with highest radioactivity in the blood, followed by the spleen, kidney, liver, lungs, and testes.	<u>Rats exposed orally:</u> Widespread, with highest radioactivity in the blood, liver, kidney, and spleen.	<u>Rats exposed orally:</u> Highest radioactivity in liver, skeletal muscle, blood, and fat (<0.1−5.4% of dose 24 hr postdosing).	<u>Mice exposed orally</u>: Highest radioactivity in serum, followed by the liver and kidney.	<u>Mice exposed orally</u>: Widespread; highest radioactivity in the plasma, followed by the liver and kidney.	<u>Rats exposed orally:</u> Highest radioactivity in liver, kidney, skin, and lungs (0.02−0.03% of dose/g tissue 96 hr postdosing).	ND

Table A-2. Comparison of Available ADME Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

2,4,6-Trinitrophenol (Picric acid) CASRN 88-89-1	**2-Methyl-4,6-dinitrophenol (DNOC) CASRN 534-52-1**	**2,4,6-Trinitrotoluene (TNT) CASRN 118-96-7**	**2,4-Dinitrophenol (2,4-DNP) CASRN 51-28-5**	**2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb) CASRN 88-85-7**	**1,3,5-Trinitrobenzene CASRN 99-35-4**	**1,3-Dinitrobenzene CASRN 99-65-0**
Metabolic pathway(s)						
• Nitro reduction • Acetylation	• Nitro reduction • Acetylation • Oxidation of methyl group • Conjugation of the hydroxyl group represents a minor pathway	• Sequential nitro reduction followed by *N*-acetylation or ring hydroxylation • Formation of hydroxylamine intermediates • Oxidation of methyl group and benzene ring represents a minor pathway	• Nitro reduction	• Nitro reduction • Oxidation of methyl group • Conjugation of some metabolites with glucuronic acid	• Nitro reduction	• Sequential nitro reduction followed by *N*-acetylation or ring hydroxylation • Conjugation of some metabolites with sulfate or glucuronic acid
Metabolites						
<u>Rats exposed orally</u> Urinary: • Parent compound (60%) • 2-Amino-4,6-dinitro-phenol (picramic acid) (18.5%) • *N*-acetylisopicramic acid (14.8%) • *N*-acetylpicramic acid (4.7%) • Unidentified components (2.4%)	<u>Rats exposed orally</u> Urinary: • Parent compound (5%) • 4,6-Diacetamido-*o*-cresol (18%) • 4,6-Dinitro-2-hydroxymethyl-phenol (4−5%) • 6-Acetamido-4-nitro-*o*-cresol (2–3%)	<u>Humans</u> Urinary: • 2-Amino-4,6-dintro-toluene • 4-Amino-2,6-dinitro-toluene • 2,4-Diamino-6-nitro-toluene • 4-Hydroxyl amino-2,6-dintro-toluene • 4-Amino-2,6-dinitro-m-cresol	<u>Humans exposed orally or via inhalation:</u> Urinary: • Parent compound • 2-Amino-4-nitro-phenol • 4-Amino-2-nitrophenol • 2,4-Diamino-phenol	<u>Rats exposed orally</u> Urinary: • 2-(2-Hydroxy-1-methylpropyl)-4,6-dinitrophenol • 2-Methyl-2-(2-hydroxy-3,5-dinitro-phenyl) propionic acid • 2-Amino-6-(1-methylpropyl)-4-nitrophenol • Glucuronide-conjugated metabolites	<u>Rats exposed orally</u> Urinary: • 3,5-Dinitroaniline • 1,3-Diamino-5-nitro-benzene • 1,3,5-Triamino-benzene Fecal: • 1,3-Diamino-5-nitro-benzene • 1,3,5-Triamino-benzene	<u>Rats exposed orally</u> Urinary: • 3-Aminoacetanilide (22%) • 4-Acetamido phenyl sulfate (6%) • 1,4-Diacetamido benzene (7%) • 3-Nitroaniline-*N*-glucuronide (4%)

Table A-2. Comparison of Available ADME Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

2,4,6-Trinitrophenol (Picric acid) CASRN 88-89-1	**2-Methyl-4,6-dinitrophenol (DNOC) CASRN 534-52-1**	**2,4,6-Trinitrotoluene (TNT) CASRN 118-96-7**	**2,4-Dinitrophenol (2,4-DNP) CASRN 51-28-5**	**2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb) CASRN 88-85-7**	**1,3,5-Trinitrobenzene CASRN 99-35-4**	**1,3-Dinitrobenzene CASRN 99-65-0**
Continued:	Continued: • 4-Acetamido-6-nitro-*o*-cresol (1–2%) • 6-Amino-4-nitro-*o*-cresol (1–2%) • Unidentified metabolites and conjugates Similar metabolites in rabbit	Continued: Similar metabolites identified in rat, mouse, rabbit, and dog urine.	Continued: <u>Rats exposed orally</u> Urinary: • Nitrophenols • 2-Amino-4-nitro-phenol <u>Rabbits exposed orally</u> Urinary: • 2,4-Diamino-phenol <u>Mice exposed orally</u> Plasma: • 2-Amino-4-nitrophenol • 4-Amino-2-nitrophenol	Continued:	Continued:	Continued: <u>Rabbits exposed orally</u> Urinary: • 3-Nitroaniline and 1,3-benzene diamine (35%) • 2,4-Diaminophenol (31%) • 2-Amino-4-nitro-phenol (14%) • 4-Amino-2-nitro-phenol (2%) • 30% of the metabolites were conjugated with glucuronic acid and 6% with sulfate

Table A-2. Comparison of Available ADME Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues						
2,4,6-Trinitrophenol (Picric acid) CASRN 88-89-1	**2-Methyl-4,6-dinitrophenol (DNOC) CASRN 534-52-1**	**2,4,6-Trinitrotoluene (TNT) CASRN 118-96-7**	**2,4-Dinitrophenol (2,4-DNP) CASRN 51-28-5**	**2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb) CASRN 88-85-7**	**1,3,5-Trinitrobenzene CASRN 99-35-4**	**1,3-Dinitrobenzene CASRN 99-65-0**
Excretory pattern						
Rats exposed orally (% dose at 24 hr): • Urine: 50.55 ± 17.97 • Feces: 6.19 ± 4.18 • Expired air: 0.14 ± 0.09	Rat exposed orally (% dose): • Urine: 29–41 • Feces: 10–23 Rabbits exposed orally (% dose): • Urine: 25–38	Rats, mice, dogs, and rabbits exposed orally (% dose at 24 hr): • Urine: 59–74	Urine is the primary elimination pathway in humans and animals.	Rats exposed orally (% dose in 72 hr): • Urine:60–65% • Feces: 25–30% Mice exposed orally (% dose in 72 hr): • Urine:35–40% • Feces: 35–40%	Rats exposed orally (% dose): • Urine: 21–36 (in 4 d) • Feces: 4 (in 4 d) • Expired air: 3–5 (in 2 d)	Rabbits exposed orally (% dose in 2 d): • Urine: 81 • Feces: 0.3–5.2 • Expired air: ND
Wyman et al. (1992)	ATSDR (2018)	ATSDR (1995b)	ATSDR (1995c)	Gibson and Rao (1973); Bandal and Casida (1972); Hathway (1970)	U.S. EPA (1997); ATSDR (1995a);	U.S. EPA (2006); ATSDR (1995a); Cossum and Rickert (1985)

ADME = absorption, distribution, metabolism, and excretion; ND = no data.

Toxicity-Like Analogues

Table A-3 summarizes available acute lethality and repeated-dose toxicity data for picric acid and the six structurally similar chemicals identified as potential analogues. Comparison of oral acute toxicity studies in rats suggests that 1,3,5-trinitrobenzene and 2,4,6-trinitrotoluene have comparable median lethal dose (LD_{50}) values to picric acid. The other candidate analogues seem to be more potent than picric acid. Clinical signs were suggestive of central nervous system (CNS) effects for picric acid and the candidate analogues with data.

As presented in the main PPRTV document, after a 28-day administration, picric acid exposure has been shown to result in liver, male reproductive, hematological, and splenic effects. Increased absolute spleen weight was identified as the critical effect. The increased spleen weight is considered a pathological consequence associated with hematological effects (increased Ret, decreased RBC and Hb), which is supported by extramedullary hematopoiesis observed in the spleen. Therefore, similar hematological and associated splenic effects were expected from the potential analogues, preferably from rat toxicity studies, the animal species tested for picric acid.

Out of the six potential analogues, 2-methyl-4,6-dinitrophenol and 2,4-dinitrophenol resulted in significantly decreased body weight in rats starting at doses of 17.3 and 46 mg/kg-day (subchronic studies), with no hematological effects at doses up to 44.9 and 182 mg/kg-day, respectively. These observations contrasted with the decreased Hb and RBC in rats treated with picric acid at a dose of 100 mg/kg-day, with no significant effect in body weight (see Table A-3). Therefore, these two chemicals were not considered toxicity-like analogues. Based on the available toxicity information from chronic studies, the critical effect of 2-(1-methylpropyl)-4,6-dinitrophenol is decreased fetal weight with a LOAEL of 1 mg/kg-day from a three-generation reproductive study in rats (U.S. EPA, 1989a). In a 2-year feeding study in mice [Dow Chemical Co. (1989) as cited in U.S. EPA (1989a)], cystic endometrial hyperplasia and testicular atrophy/degeneration with hypospermatogenesis were observed at all doses (1, 3, and 10 mg/kg-day); lenticular opacities were observed at 3 and 10 mg/kg-day (low-dose animals not examined) (U.S. EPA, 1989a). It is unclear from U.S. EPA (1989a) whether hematological effects were evaluated in this study, and the original report is not readily available. Further, no systemic toxicity studies were conducted in rats and no toxicity information is available with respect to hematological and splenic effects at doses greater than 10 mg/kg-day in mice. Therefore, due to limited toxicity information for comparison purposes, 2-(1-methylpropyl)-4,6-dinitrophenol was not considered a toxicity-like analogue of picric acid (see Table A-3).

IRIS assessments for 1,3,5-trinitrobenzene and 1,3-dinitrobenzene have identified hematological and splenic effects in rats (U.S. EPA, 1997, 1988a) (see Table A-3). Therefore, 1,3,5-trinitrobenzene and 1,3-dinitrobenzene were considered toxicity-like analogues.

Table A-3. Comparison of Available Repeated-Dose Toxicity Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

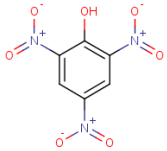
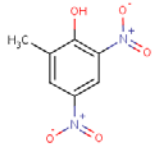
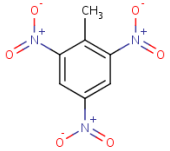
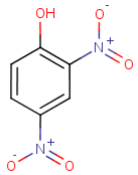
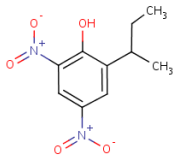
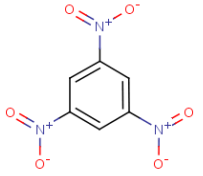
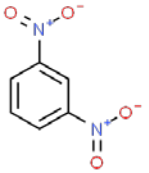
Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitro-toluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene
Structure							
CASRN	88-89-1	534-52-1	118-96-7	51-28-5	88-85-7	99-35-4	99-65-0
Acute lethality studies[a]							
Rat oral LD_{50} (mg/kg)	200−629	7	607	30	25	275	59.5
Effect	Ataxia, tremors, convulsions, circumorbital discharge (chromodactorea) of the eyes	NV	Respiratory stimulation, changes in urine composition, infammation, and necrosis of the bladder	NV	Depressed behavioral activity, convulsions or effect on seisure threshold, and respiratory stimulation	Dyspnea, rigidity, and depressed behavioral activity	Dyspnea, depressed behavioral activity, and effect on skin and appendages
Short-term or subchronic treatment (oral)							
Subchronic RfD (mg/kg-d)	1×10^{-2}	8×10^{-4}	NV	2×10^{-2}	NV	NV	NV
Critical effects	Increased absolute spleen weight	Reduced body weight, excessive perspiration and fatigue, elevated basal metabolic rate and body temperature, as well as ocular effects (based on human study)	Increased liver weight, change of liver enzymes, and liver lesions (26-wk study in dogs)	Cataract formation (human study)	NV	NV	Increased spleen weight (16-wk study in rats)

Table A-3. Comparison of Available Repeated-Dose Toxicity Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitro-toluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene
Other effects	Hematological and related splenic effects (hematopoiesis), increased liver weight, and testicular effects	(1) Decreased body weight; no hematological effects were specified at doses up to 44.9 mg/kg-d (evaluated hematological parameters included RBC, WBC, and Hb; 182-d oral study in rats). (2) Decreased blood pyruvate, T3, and T4 levels; no hematological toxicity was specified at doses up to 41.0 mg/kg-d (examined hematological endpoints included RBC, Hb, MCH, MCV, and WBC; 90-d oral study in rats). (3) Increased percentages of abnormal sperm (reproductive	Comprehensive hematological parameters were evaluated, but it is unclear whether those effects were observed at doses up to 32 mg/kg-d (26-wk study in dogs). No information with respect to hematological effects in rats was available in IRIS risk assessment. However, toxic effects on hematologic parameters and related splenic effects were observed in other subchronic studies in rats, mice, and dogs at doses higher than those causing liver effects as described in	(1) No effects were observed at doses up to 10 mg/kg-d (free-standing NOAEL; hematological endpoints were examined; 27-wk study in dogs). (2) Decreased body weight (<10%), slight liver, kidney, spleen (congestion and hemosiderosis), and testicular atrophy at a dose of 46-mg/kg-d. No hematological effects were observed at doses up to 182 mg/kg-d. (Hematological examination, including RBC and Hb; 6-mo study in rats.) (3) In a similar study to the picric acid study by Koizumi et al. (2001), young rats	NV	Methemoglobi-nemia and spleen-erythroid cell hyperplasia; increased relative spleen and liver weight; and decreased testis weight (90- and 180-d interim sacrifice in a 2-yr chronic study in rats)	Decreased body-weight gain, decreased Hb, testicular atrophy, and splenic hemosiderin

Table A-3. Comparison of Available Repeated-Dose Toxicity Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitro-toluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene
Continued:	Continued:	Continued: study in male rats).	Continued: ATSDR (1995b) risk assessment (p. 46/208).	Continued: were tested for behavior, hematological, urinalysis, biochemistry, organ weight, and histopathology. Decreased locomotor activity and salivation were observed at a dose of 80 mg/kg-d. No hematological, liver, spleen, or testicular effects were observed (28-d study in rats).	Continued:	Continued:	Continued:
POD (mg/kg-d)	$BMDL_{1SD}$ of 17.3	LOAEL of 0.8	NV	NV	NV	NV	NV
UF_C	300	1,000	NV	NV	NV	NV	NV
Source	Subchronic p-RfC in this assessment	U.S. EPA (2010)	U.S. EPA (1989b)	U.S. EPA (1991); U.S. EPA (2007)	NV	U.S. EPA (1997)	U.S. EPA (1988a)

Table A-3. Comparison of Available Repeated-Dose Toxicity Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitro-toluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene
Chronic treatment (oral)							
Chronic RfD (mg/kg-d)	NA	8×10^{-5}	5×10^{-4}	2×10^{-3}	1×10^{-3}	3×10^{-2}	1×10^{-4}
Critical effects	NV	NV	IRIS summary does not specify toxic effects at doses >0.4 mg/kg-d (DOD, 1984) (2-yr study in rats)	NV	Decreased fetal weight (3-generation reproductive study in rats)	Methemoglobi-nemia and spleen-erythroid cell hyperplasia (2-yr study in rats)	NV
Other effects (oral)	NV	NV		Decreases in body weight at doses >47 mg/kg-d. No treatment-related effects in histopathology at doses up to 187 mg/kg-d. It is unclear whether hematology parameters were evaluated and what tissues/organs were evaluated pathologically (2-yr study in rats).	Cystic endometrial hyperplasia and testicular atrophy with hypospermatogenesis at doses ≥1 mg/kg-d and lenticular opacities at doses of 3 and 10 mg/kg-d. It is unclear if hematological effects were evaluated. It is unclear if Dinoseb causes hematological, splenic, or testicular effects.		NV

Table A-3. Comparison of Available Repeated-Dose Toxicity Data for Picric Acid (CASRN 88-89-1) and Candidate Analogues

Type of Data	2,4,6-Trinitrophenol (Picric Acid)	2-Methyl-4,6-dinitrophenol (DNOC)	2,4,6-Trinitro-toluene (TNT)	2,4-Dinitrophenol (2,4-DNP)	2-(1-Methylpropyl)-4,6-dinitrophenol (Dinoseb)	1,3,5-Trinitro-benzene	1,3-Dinitrobenzene
POD (mg/kg-d)	NV	LOAEL: 0.8	LOAEL: 0.5	LOAEL: 2	LOAEL: 1	NOAEL: 2.68	LOAEL: 0.4
UF_C	NV	10,000	1,000	1,000	1,000	100	3,000
Source	NV	U.S. EPA (2010)	U.S. EPA (1989b)	U.S. EPA (1991); U.S. EPA (2007)	U.S. EPA (1989a)	U.S. EPA (1997)	U.S. EPA (1988a)

[a]Data for picric acid from Table 4B in main body of this report; data for candidate analogues from ChemIDplus (2018).

BMDL = benchmark dose lower confidence limit; Hb = hemoglobin; IRIS = Integrated Risk Information System; LD_{50} = median lethal dose; LOAEL = lowest-observed-adverse-effect level; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; NA = not applicable; NOAEL = no-observed-adverse-effect level; NV = not available; POD = point of departure; p-RfC = provisional reference concentration; RBC = red blood cell (erythrocyte); RfD = oral reference dose; SD = standard deviation; T3 = triiodothyronine; T4 = thyroxine; UF_C = composite uncertainty factor; WBC = white blood cell (leukocyte).

For 2,4,6-trinitrotoluene, liver effects (increased liver weight, alterations in liver enzyme levels, and liver lesions) were identified as critical effects with a LOAEL of 0.5 mg/kg-day, based on a 26-week study in dogs (U.S. EPA, 1989b). According to the IRIS assessment, comprehensive endpoints including clinical chemistry, hematological evaluation, urinalyses, periodic electrocardiography (ECG), and ophthalmic examinations were evaluated in this study, but it is unclear whether hematological and splenic effects were observed at this dose or higher. No information on 2,4,6-trinitrotoluene with respect to hematological effects in rats was available in the IRIS risk assessment (U.S. EPA, 1989b). However, the effects of 2,4,6-trinitrotoluene in the hematological and splenic compartments were observed in other subchronic studies in rats, mice, and dogs at doses higher than the dose that caused liver effects as described in ATSDR (1995b). Thus, 2,4,6-trinitrotoluene is also considered a toxicity-like analogue.

In conclusion, an attempt was made to identify a suitable analogue to derive chronic toxicity values for picric acid. Comparison of the potential analogues (2-methyl-4,6-dinitro-phenol; 2,4-dinitrophenol; 2,4,6-trinitrotoluene; 2-[1-methylpropyl]-4,6-dinitrophenol; 1,3,5-trinitrobenzene; and 1,3-dinitrobenzene) was made based on their profiles of structural similarity, metabolic profile, and tissue-specific toxicity, and 2,4,6-trinitrotoluene; 1,3,5-trinitrobenzene; and 1,3-dinitrobenzene were kept for the final selection.

Weight-of-Evidence Approach

To select the best analogue chemical for picric acid, the following considerations were used in a WOE approach: (1) lines of evidence from U.S. EPA assessments are preferred; (2) chemicals that have chronic toxicity information are preferred; and (3) if there are no clear indications as to the best analogue chemical based on the first two considerations, then the candidate analogue with the highest structural similarity may be preferred.

Overall, based on the WOE of all the information presented above, 1,3,5-trinitrobenzene seems to be the most appropriate analogue for picric acid because of the following factors:

1) U.S. EPA IRIS identified that the critical effect of 1,3,5-trinitrobenzene is "methemoglobinemia and spleen-erythroid cell hyperplasia," which is consistent with the hematological and associated splenic effects observed in rats treated with picric acid.
2) The critical effect for 1,3,5-trinitrobenzene is based on a 2-year chronic study in rats (compared to PODs based on subchronic studies for 2,4,6-trinitrotoluene and 1,3-dinitrobenzene IRIS assessments).
3) Relatively high structural similarity scores of 75.03 and 73.6% were found using the NLM's ChemIDplus database (ChemIDplus, 2018) and the U.S. EPA DSSTox database, respectively.
4) Lethality studies in rats suggest that 1,3,5-trinitrobenzene and picric acid have similar potencies (oral LD_{50}s), and their acute toxic effects primarily target the CNS.

The 1,3,5-trinitrobenzene IRIS summary (U.S. EPA, 1997) cited Reddy et al. (2001), Reddy et al. (1997), and Reddy et al. (1996) as the principal studies for the reference dose (RfD):

"Chronic toxic effects of 1,3,5-TNB in male and female Fischer 344 rats were evaluated by feeding powdered certified laboratory chow diet supplemented with varied concentrations of TNB for 2 years. Based on food consumption, the average TNB intake was calculated for both males and females.

"The study was conducted in accordance with the U.S. EPA guidelines for chronic toxicity studies as required by the GLP standards. One of the unique features of this study is that 10 animals/sex were sacrificed at the end of 90 days, 6 months and 1 year, and 25 or more rats were sacrificed at 2 years; complete toxicological evaluations were performed during these periods.

"High-dose animals showed decreased body weight gains associated with decreased food consumption. Relative organ weight changes for the brain (increase), spleen (increase), liver (increase) and testes (decrease in 90- and 180-day periods) were reported for all treated animals dosed with TNB at levels higher than 3 mg/kg/day; adverse hematological findings (decreased hematocrit and hemoglobin) and increased methemoglobulin [sic] *were consistently reported in all animals treated at these levels. Histopathological findings in the 1-year study revealed extramedullary hematopoiesis in rats treated with TNB at doses of 3 mg/kg-day or higher. In the 2-year study, these effects were seen only in rats dosed with TNB at the high dosage level (13.23 mg/kg/day). The adverse effects, such as increased methemoglobin, erythroid cell hyperplasia, and increased relative organ weights, observed during interim sacrifices in rats receiving 60 ppm TNB did not persist and were not detected in rats fed 60 ppm TNB for 2 years, suggesting that an adaptive mechanism has taken place in order to compensate adverse effects observed during interim sacrifices.*

"Results of this study exhibited clear evidence of toxicity of the hematopoietic system as has been reported for other nitroaroniatics [sic] *such as, dinitrobenzene and trinitrotoluene. The NOAEL for this study is 2.68 mg/kg/day and the LOAEL for hematological effects is 13.31 mg/kg/day."*

ORAL TOXICITY VALUES

Derivation of Screening Chronic Provisional Reference Dose

Based on the overall analogue approach presented in this PPRTV assessment, the critical effects identified in the IRIS assessment (U.S. EPA, 1997) of methemoglobinemia and spleen-erythroid cell hyperplasia for 1,3,5-trinitrobenzene established in F344 rats from a 2-year study (Reddy et al., 2001; Reddy et al., 1997) are identified as the potential analogue critical effects for picric acid. The NOAEL of 2.68 mg/kg-day identified in female rats exposed to 1,3,5-trinitrobenzene is selected as the POD for derivation of the chronic p-RfD for picric acid.

As described in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the POD of 2.68 mg/kg-day is converted to an HED through an application of a DAF derived as follows:

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

where

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

Using a reference BW_a of 0.229 kg for female F344 rats and a default BW_h of 70 kg for humans (U.S. EPA, 1988b), the resulting DAF is 0.24. Applying this DAF to the NOAEL identified in the rat study yields an analogue POD (HED) as follows:

Analogue POD (HED) = NOAEL (mg/kg-day) × DAF
= NOAEL (mg/kg-day) × 0.24
= 2.68 mg/kg-day × 0.24
= 0.643 mg/kg-day

The POD based on 1,3,5-trinitrobenzene as an analogue of picric acid is considered applicable to both picric acid and ammonium picrate for reasons described in the "Introduction" section. Both are equivalent sources of picrate in the environment and in the body. Furthermore, the analogue chemical analysis presented here shows that the spectrum of effects associated with picric acid (primarily hematological, splenic, and testicular effects) is consistent with the structurally related chemicals considered as analogues. These chemicals also all share a common primary metabolic pathway (i.e., nitro reduction). The findings link the picrate anion to the effects observed after dosing with picric acid. Because the same picrate anion is formed from ammonium picrate, and in the same amounts, confidence is high that the conclusions made in the PPRTV assessment for picric acid (based on the picrate anion) are applicable to ammonium picrate as well.

To derive the screening chronic p-RfD for picric acid and ammonium picrate, a composite uncertainty factor (UF_C) of 300 (see Table A-4) was applied to the analogue POD (HED). As described in Wang et al. (2012) the uncertainty factors typically applied to the chemical of concern are the same as those applied to the analogue unless additional information is available. In this case, the interspecies uncertainty factor (UF_A) for picric acid was reduced from 10 to 3 due to the conversion of the POD from an animal dose to an HED [the IRIS assessment for the 1,3,5-trinitrobenzene was performed prior to the recommended use of $BW^{3/4}$ scaling for noncancer effects (U.S. EPA, 2011b)]. Further, the database uncertainty factor (UF_D) for picric acid was raised from 1 to 10 to account for limited information with regard to reproductive toxicity and no information with regard to developmental toxicity for picric acid [reproductive and developmental toxicity data for 1,3,5-trinitrobenzene were available and indicated that these were not sensitive endpoints for that chemical (U.S. EPA, 2011b)].

The screening chronic p-RfD for picric acid and ammonium picrate is derived as follows:

Screening Chronic p-RfD = Analogue POD (HED) ÷ UF_C
= 0.643 mg/kg-day ÷ 300
= **2×10^{-3} mg/kg-day**

Table A-4. Uncertainty Factors for the Screening Chronic p-RfD for Picric Acid (CASRN 88-89-1) and Ammonium Picrate (CASRN 131-74-8)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for residual uncertainty, including toxicodynamic differences between rats and humans following oral picrate exposure. The toxicokinetic uncertainty has been accounted for by calculation of an HED by applying a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 is applied based on unknown and unaccountable database deficiencies of picric acid and ammonium picrate. For the analogue chemical, systemic toxicity seems to be more sensitive than developmental and reproductive effects.
UF_H	10	A UF_H of 10 is applied to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of picric acid or ammonium picrate in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because a chronic study was selected as the principal study.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; 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.

APPENDIX B. DATA TABLES

Table B-1. Body and Organ Weight for Newborn S-D Rats in Dose-Finding Study Exposed to Picric Acid (CASRN 88-89-1) for 14 Days (PNDs 4−17)[a, b]

Dose (mg/kg-d)[c]	0	16.3	81.4
Male			
Number of animals	4	4	3
Body weight (g)	48.9 ± 3.7	47.7 ± 2.6 (−2.5%)	42.3 ± 2.0* (−13%) [d]
Absolute liver weight (g)	1.73 ± 0.14	1.67 ± 0.13 (−3.5%)	1.70 ± 0.13 (−1.7%)
Relative liver weight (g/100 g BW)	3.55 ± 0.10	3.49 ± 0.12 (−1.7%)	4.01 ± 0.13** (13%) [d]
Absolute spleen weight (g)	0.21 ± 0.04	0.21 ± 0.02 (0%)	0.17 ± 0.01 (−19%)
Relative spleen weight (g/100 g BW)	0.44 ± 0.07	0.45 ± 0.05 (2.3%)	0.40 ± 0.03 (−9.1%)
Absolute kidney weight (g)	0.58 ± 0.03	0.56 ± 0.04 (−3.4%)	0.43 ± 0.05** (−26%)
Relative kidney weight (g/100 g BW)	1.18 ± 0.04	1.17 ± 0.05 (−0.8%)	1.02 ± 0.08** (−14%)
Female			
Number of animals	4	4	2
Body weight (g)	45.2 ± 2.2	47.5 ± 3.1 (5.1%)	38.6 (−15%) [d]
Absolute liver weight (g)	1.57 ± 0.08	1.72 ± 0.09 (9.6%)	1.64 (4.5%)
Relative liver weight (g/100 g BW)	3.48 ± 0.25	3.62 ± 0.10 (4.0%)	4.23 (22%) [d]
Absolute spleen weight (g)	0.20 ± 0.03	0.20 ± 0.04 (0%)	0.17 (−15%)
Relative spleen weight (g/100 g BW)	0.43 ± 0.04	0.43 ± 0.06 (0%)	0.44 (2.3%)
Absolute kidney weight (g)	0.55 ± 0.02	0.57 ± 0.05 (3.6%)	0.43 (−22%)
Relative kidney weight (g/100 g BW)	1.22 ± 0.06	1.20 ± 0.06 (−1.6%)	1.12 (−8.2%)

[a]Takahashi et al. (2004).
[b]Values are mean ± SD (percent change compared with control); percent change control = [(treatment mean – control mean) ÷ control mean] × 100.
[c]All animals in the 407-mg/kg-day dose group died by Day 4; therefore, data from this dose group are excluded from this table.
[d]Not statistically significant but biologically relevant (≥10% increase).
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

BW = body weight; PND = postnatal day; SD = standard deviation; S-D = Sprague-Dawley.

Table B-2. Body and Organ Weight for Newborn S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 18 Days (PNDs 4–21)[a, b]

Dose (mg/kg-d)	0	4.1	16.3	65.1
Male				
Number of animals	6	6	6	6
Body weight (g)	63.4 ± 4.9	63.0 ± 2.8 (−1%)	63.7 ± 5.7 (0%)	61.8 ± 4.8 (−3%)
Absolute liver weight (g)	2.69 ± 0.22	2.74 ± 0.14 (2%)	2.79 ± 0.24 (4%)	2.97 ± 0.38 (10%) [c]
Relative liver weight (g/100 g BW)	4.25 ± 0.16	4.35 ± 0.12 (2%)	4.38 ± 0.08 (3%)	4.79 ± 0.28** (13%) [c]
Absolute spleen weight (g)	0.34 ± 0.07	0.35 ± 0.06 (3%)	0.38 ± 0.04 (12%)	0.37 ± 0.06 (9%)
Relative spleen weight (g/100 g BW)	0.54 ± 0.07	0.56 ± 0.08 (4%)	0.60 ± 0.05 (11%)	0.60 ± 0.05 (11%)
Absolute kidney weight (g)	0.74 ± 0.12	0.73 ± 0.08 (−1%)	0.77 ± 0.03 (4%)	0.73 ± 0.12 (−1%)
Relative kidney weight (g/100 g BW)	1.16 ± 0.12	1.16 ± 0.09 (0%)	1.21 ± 0.10 (4%)	1.18 ± 0.12 (2%)
Absolute epididymis weight (mg)	57.6 ± 4.6	55.4 ± 6.0	57.6 ± 7.3	50.3 ± 3.7*
Relative epididymis weight (mg/100 g BW)	91.1 ± 6.9	87.9 ± 7.2	91.3 ± 16.4	81.9 ± 7.9
Absolute testis weight (mg)	326 ± 47	302 ± 27	319 ± 22	295 ± 20
Relative testis weight (mg/100 g BW)	513 ± 54	479 ± 26	504 ± 44	478 ± 27

Table B-2. Body and Organ Weight for Newborn S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 18 Days (PNDs 4–21)[a, b]

Dose (mg/kg-d)	0	4.1	16.3	65.1
Female				
Number of animals	6	6	6	6
Body weight (g)	59.0 ± 3.3	59.6 ± 2.3 (1%)	57.0 ± 4.6 (−3%)	58.8 ± 5.3 (−2%)
Absolute liver weight (g)	2.46 ± 0.22	2.44 ± 0.24 (−1%)	2.33 ± 0.25 (−5%)	2.75 ± 0.28 (12%) [c]
Relative liver weight (g/100 g BW)	4.18 ± 0.35	4.09 ± 0.29 (−2%)	4.09 ± 0.19 (−2%)	4.67 ± 0.19* (12%)
Absolute spleen weight (g)	0.32 ± 0.04	0.33 ± 0.04 (3%)	0.29 ± 0.05 (−9%)	0.37 ± 0.05 (16%)
Relative spleen weight (g/100 g BW)	0.54 ± 0.05	0.55 ± 0.07 (2%)	0.51 ± 0.08 (−6%)	0.62 ± 0.03** (15%)
Absolute kidney weight (g)	0.69 ± 0.05	0.69 ± 0.06 (0%)	0.66 ± 0.06 (−4%)	0.70 ± 0.05 (1%)
Relative kidney weight (g/100 g BW)	1.17 ± 0.09	1.16 ± 0.08 (−1%)	1.16 ± 0.10 (−1%)	1.20 ± 0.06 (3%)

[a]Takahashi et al. (2004).
[b]Values are mean ± SD (percent change compared with control); percent change control = [(treatment mean – control mean) ÷ control mean] × 100.
[c]Not statistically significant but biologically relevant (≥10% increase).
*Significant difference from control at $p < 0.05$.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

BW = body weight; PND = postnatal day; SD = standard deviation; S-D = Sprague-Dawley.

Table B-3. Body and Organ Weight for Young S-D Rats in Dose-Finding Study Exposed to Picric Acid (CASRN 88-89-1) for 14 Days[a, b]

ADD (mg/kg-d)[c]	0	20	100
Male			
HED (mg/kg-d)	0	4.9	25.1
Number of animals	3	3	3
Body weight (g)	267 ± 15	257 ± 7 (−3.7%)	276 ± 9 (3.4%)
Absolute liver weight (g)	10.8 ± 0.4	10.9 ± 0.7 (0.9%)	12.2 ± 0.2* (13%) [d]
Relative liver weight (g/100 g BW)	4.04 ± 0.12	4.26 ± 0.39 (5.4%)	4.43 ± 0.17 (9.7%)
Absolute spleen weight (g)	0.77 ± 0.10	0.75 ± 0.03 (−2.6%)	0.91 ± 0.07 (18%)
Relative spleen weight (g/100 g BW)	0.29 ± 0.03	0.29 ± 0.02 (0%)	0.33 ± 0.02* (14%)
Absolute kidney weight (g)	2.29 ± 0.25	2.12 ± 0.16 (−7.4%)	2.39 ± 0.12 (4.4%)
Relative kidney weight (g/100 g BW)	0.86 ± 0.06	0.83 ± 0.04 (−3.5%)	0.87 ± 0.02 (1.2%)
Female			
HED (mg/kg-d)	0	4.5	22.4
Number of animals	3	3	3
Body weight (g)	165 ± 9	172 ± 4 (4.2%)	175 ± 8 (6.1%)
Absolute liver weight (g)	6.4 ± 0.8	6.7 ± 0.1 (4.7%)	8.0 ± 0.6* (25%) [d]
Relative liver weight (g/100 g BW)	3.85 ± 0.28	3.90 ± 0.07 (1.3%)	4.54 ± 0.23* (18%) [d]
Absolute spleen weight (g)	0.49 ± 0.13	0.45 ± 0.13 (−8.2%)	0.56 ± 0.05 (14%)
Relative spleen weight (g/100 g BW)	0.30 ± 0.06	0.26 ± 0.08 (−13%)	0.32 ± 0.01 (6.7%)
Absolute kidney weight (g)	1.40 ± 0.03	1.45 ± 0.13 (3.6%)	1.52 ± 0.17 (8.6%)
Relative kidney weight (g/100 g BW)	0.85 ± 0.03	0.84 ± 0.07 (−1.2%)	0.87 ± 11 (2.4%)

[a]Takahashi et al. (2004).
[b]Values are mean ± SD (percent change compared with control); percent change control = [(treatment mean – control mean) ÷ control mean] × 100.
[c]Most animals in the 500-mg/kg-day dose group died by Day 2; therefore, data from this dose group are excluded from this table.
[d]Not statistically significant but biologically relevant (≥10% increase).
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

ADD = adjusted daily dose; BW = body weight; HED = human equivalent dose; SD = standard deviation; S-D = Sprague-Dawley.

Table B-4. Hematological Parameters for Young S-D Rats in Dose-Finding Study Exposed to Picric Acid (CASRN 88-89-1) for 14 Days[a, b]

ADD (mg/kg-d)	0	20	100
Male			
HED (mg/kg-d)	0	4.9	25.1
Number of animals	3	3	3
WBC (× 10^2/mL)	117 ± 26	94 ± 20	108 ± 21
RBC (× 10^4/mL)	682 ± 13	651 ± 24	646 ± 32
Hb (g/dL)	14.0 ± 0.6	13.8 ± 0.2	13.8 ± 0.6
Hct (%)	40.9 ± 1.4	41.3 ± 1.5	40.9 ± 2.7
MCV (fL)	60.0 ± 3.1	63.4 ± 1.1	63.3 ± 1.7
MCHC (%)	34.2 ± 0.3	33.5 ± 1.0	33.7 ± 0.9
Ret (‰)	59.8 ± 5.6	61.1 ± 3.7	72.6 ± 8.2
Female			
HED (mg/kg-d)	0	4.5	22.4
Number of animals	3	3	3
WBC (× 10^2/mL)	82 ± 7	70 ± 12	98 ± 31
RBC (× 10^4/mL)	711 ± 6	690 ± 31	639 ± 47
Hb (g/dL)	14.6 ± 0.1	14.5 ± 0.3	13.5 ± 0.7*
Hct (%)	42.4 ± 0.3	41.4 ± 0.6	38.5 ± 1.7**
MCV (fL)[c]	59.6 ± 0.8	60.0 ± 3.6	60.3 ± 1.8
MCHC (%)	34.5 ± 0.4	35.2 ± 0.3	35.0 ± 0.3
Ret (‰)	37.6 ± 1.5	39.6 ± 6.9	56.3 ± 3.6**

[a]Takahashi et al. (2004).
[b]Values are mean ± SD.
[c]Femtoliters (10^{-15} L).
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

ADD = adjusted daily dose; Hb = hemoglobin levels; Hct = hematocrit levels; HED = human equivalent dose; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell (erythrocyte); Ret = reticulocyte; SD = standard deviation; S-D = Sprague-Dawley; WBC = total white blood cell (leukocyte).

Table B-5. Hematological Parameters for Young S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 28 Days[a, b]

ADD (mg/kg-d)	0	4	20	100
Male				
HED (mg/kg-d)	0	1.1	5.4	26.9
Number of animals	6	6	6	6
WBC (× 10^2/mL)	93 ± 14	98 ± 14	112 ± 22	146 ± 38**
RBC (× 10^4/mL)	720 ± 32	720 ± 13	739 ± 34	661 ± 52*
Hb (g/dL)	14.3 ± 0.3	14.6 ± 0.5	14.8 ± 0.7	13.4 ± 0.7*
Hct (%)	40.9 ± 1.0	41.5 ± 1.8	42.6 ± 1.4	39.1 ± 2.2
MCV (fL)	56.8 ± 1.6	57.7 ± 2.3	57.8 ± 2.3	59.3 ± 2.7
MCHC (%)	35.0 ± 0.7	35.2 ± 0.6	34.8 ± 0.6	34.1 ± 0.5
Ret (‰)	31.4 ± 1.4	29.8 ± 4.1	31.6 ± 3.8	54.7 ± 7.6**
Female				
HED (mg/kg-d)	0	1.0	4.8	24.0
Number of animals	6	6	6	6
WBC (× 10^2/mL)	67 ± 18	79 ± 27	73 ± 15	123 ± 33**
RBC (× 10^4/mL)	706 ± 30	711 ± 47	713 ± 41	608 ± 19**
Hb (g/dL)	14.2 ± 0.5	14.3 ± 0.5	14.3 ± 0.6	12.6 ± 0.3**
Hct (%)	39.3 ± 1.2	40.3 ± 1.9	40.3 ± 1.8	37.3 ± 0.9
MCV (fL)[c]	55.8 ± 0.9	56.9 ± 3.4	56.6 ± 1.7	61.4 ± 2.4**
MCHC (%)	36.2 ± 0.9	35.6 ± 0.6	35.6 ± 0.7	33.9 ± 0.3**
Ret (‰)	25.5 ± 4.6	25.2 ± 1.0	24.1 ± 3.3	65.5 ± 5.9*

[a]Takahashi et al. (2004).
[b]Values are mean ± SD.
[c]Femtoliters (10^{-15} L).
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

ADD = adjusted daily dose; Hb = hemoglobin levels; Hct = hematocrit levels; HED = human equivalent dose; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell (erythrocyte); Ret = reticulocyte; SD = standard deviation; S-D = Sprague-Dawley; WBC = total white blood cell (leukocyte).

Table B-6. Body and Organ Weight for Young S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 28 Days[a, b]

ADD (mg/kg-d)	0	4	20	100
Male				
HED (mg/kg-d)	0	1.1	5.4	26.9
Number of animals	6	6	6	6
Body weight (g)	374 ± 12	380 ± 31 (2%)	384 ± 35 (3%)	367 ± 27 (−2%)
Absolute liver weight (g)	14.2 ± 1.3	14.0 ± 0.9 (−1%)	14.4 ± 1.8 (1%)	15.6 ± 1.1 (10%) [d]
Relative liver weight (g/100 g BW)	3.79 ± 0.31	3.69 ± 0.19 (−3%)	3.73 ± 0.23 (−2%)	4.24 ± 0.24* (12%) [d]
Absolute spleen (g)[c]	0.82 ± 0.08	0.76 ± 0.08 (−7%)	0.89 ± 0.19 (9%)	1.18 ± 0.16** (44%)
Relative spleen weight (g/100 g BW)[c]	0.22 ± 0.02	0.20 ± 0.02 (−9%)	0.23 ± 0.03 (5%)	0.32 ± 0.03** (45%)
Absolute kidney weight (g)	2.62 ± 0.13	2.57 ± 0.13 (−2%)	2.81 ± 0.33 (7%)	2.72 ± 0.13 (4%)
Relative kidney weight (g/100 g BW)	0.70 ± 0.03	0.68 ± 0.05 (−3%)	0.73 ± 0.06 (4%)	0.74 ± 0.03 (6%)
Absolute testis weight (g)	3.08 ± 0.32	3.09 ± 0.19	3.13 ± 0.25	3.29 ± 0.35
Relative testis weight (g/100 g BW)	0.82 ± 0.09	0.82 ± 0.06	0.82 ± 0.05	0.90 ± 0.05
Absolute epididymis weight (g)	0.82 ± 0.06	0.78 ± 0.06 (−5%)	0.78 ± 0.07 (−5%)	0.63 ± 0.10** (−23%)
Relative epididymis weight (g/100 g BW)	0.22 ± 0.02	0.21 ± 0.02 (−5%)	0.20 ± 0.01 (−9%)	0.17 ± 0.03** (−23%)

Table B-6. Body and Organ Weight for Young S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 28 Days[a, b]

ADD (mg/kg-d)	0	4	20	100
Female				
HED (mg/kg-d)	0	1.0	4.8	24.0
Number of animals	6	6	6	6
Body weight (g)	242 ± 19	241 ± 17 (0%)	237 ± 29 (−2%)	233 ± 14 (−4%)
Absolute liver weight (g)	8.2 ± 0.7	8.0 ± 0.8 (−2%)	8.2 ± 1.5 (0%)	9.7 ± 1.2 (18%)[d]
Relative liver weight (g/100 g BW)	3.38 ± 0.11	3.32 ± 0.15 (−2%)	3.45 ± 0.19 (2%)	4.16 ± 0.27** (23%)[d]
Absolute spleen weight (g)[c]	0.51 ± 0.08	0.58 ± 0.05 (14%)	0.54 ± 0.08 (6%)	0.98 ± 0.12** (92%)
Relative spleen weight (g/100 g BW)[c]	0.21 ± 0.04	0.24 ± 0.02 (14%)	0.23 ± 0.20 (10%)	0.42 ± 0.05** (100%)
Absolute kidney weight (g)	1.77 ± 0.16	1.73 ± 0.20 (−2%)	1.67 ± 0.20 (−6%)	1.86 ± 0.17 (5%)
Relative kidney weight (g/100 g BW)	0.74 ± 0.07	0.71 ± 0.04 (−4%)	0.71 ± 0.05 (−4%)	0.80 ± 0.06 (8%)

[a]Takahashi et al. (2004).
[b]Values are mean ± SD (percent change compared with control); percent change control = [(treatment mean – control mean) ÷ control mean] × 100.
[c]Statistically significant as calculated for this review (ANOVA contrast with equally spaced coefficients); trend $p < 0.01$.
[d]Not statistically significant but biologically relevant (≥10% increase).
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

ADD = adjusted daily dose; ANOVA = analysis of variance; BW = body weight; HED = human equivalent dose; S-D = Sprague-Dawley; SD = standard deviation.

Table B-7. Histopathological Parameters for Young Male S-D Rats in Main Study Exposed to Picric Acid (CASRN 88-89-1) for 28 Days[a, b]

		ADD (HED), mg/kg-d			
		0	**4 (1.1)**	**20 (5.4)**	**100 (26.9)**
Number of animals examined	**Grade**	6	6	6	6
Spleen					
Development, germinal center	+	0	0	0	5*
Extramedullary hematopoiesis, erythrocyte	+	0	0	0	6**
Hemosiderin deposition	Total	0	0	0	4
	+	0	0	0	3
	++	0	0	0	1
Cecum					
Ulcer	Total	0	0	0	4
	+	0	0	0	1
	++	0	0	0	2
	+++	0	0	0	1
Liver					
Hypertrophy, hepatocytes, centrilobular	+	0	0	0	4
Testis					
Atrophy, seminiferous tubules, diffuse	Total	0	0	0	6**
	+	0	0	0	6**
Epididymis					
Cell debris, lumen	Total	0	0	0	4
	+	0	0	0	3
	++	0	0	0	1
Decrease in sperm	Total	0	0	0	6*
	+	0	0	0	5*
	++	0	0	0	1

[a]Takahashi et al. (2004).
[b]Grade sign: +, mild; ++, moderate; +++, marked.
*Significant difference from control at $p < 0.05$ as calculated by study authors.
**Significant difference from control at $p < 0.01$ as calculated by study authors.

ADD = adjusted daily dose; HED = human equivalent dose; S-D = Sprague-Dawley.

Table B-8. Histopathological Parameters for Young Female S-D Rats Exposed to Picric Acid (CASRN 88-89-1) for 28 Days[a, b]

		ADD (HED), mg/kg-d			
		0	**4 (1.0)**	**20 (4.8)**	**100 (24.0)**
Number of animals examined	**Grade**	6	6	6	6
Spleen					
Development, germinal center	+	0	0	0	5*
Extramedullary hematopoiesis, erythrocyte	+	0	0	0	6**
Hemosiderin deposition	Total	0	0	0	6**
	+	0	0	0	3
	++	0	0	0	3
Cecum					
Ulcer	++	0	0	0	3
Liver					
Hypertrophy, hepatocytes, centrilobular	+	0	0	0	3

[a]Takahashi et al. (2004).
[b]Grade sign: +, mild; ++, moderate; +++, marked.
*Significant difference from control at $p < 0.05$.
**Significant difference from control at $p < 0.01$.

ADD = adjusted daily dose; HED = human equivalent dose; S-D = Sprague-Dawley.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS

MODELING PROCEDURE FOR CONTINUOUS DATA

Benchmark dose (BMD) modeling of continuous data is conducted with U.S. EPA's Benchmark Dose Software (BMDS; Version 2.7). All continuous models available within the software are fit using a benchmark response (BMR) of 1 standard deviation (SD) relative risk or 10% extra risk when a biologically determined BMR is available (e.g., BMR 10% relative deviation [RD] for liver weight based on a biologically significant increase of 10%), as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). An adequate fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a homogeneous variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit (BMDL) was selected if the BMDLs estimated from different models varied greater than threefold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) from which to derive the provisional reference dose (p-RfD).

Table C-1. Summary of BMD Modeling of Data from Newborn S-D Rats Treated with Picric Acid (CASRN 88-89-1) via Gavage for 18 Days[a]

Endpoint	Sex	Model	*p*-Value[b]	*p*-Value Test 2	*p*-Value Test 3	AIC for Fitted Model	Scaled Residual	BMD (mg/kg-d)	BMDL (mg/kg-d)
Increased absolute liver weight	M	Linear	0.9606	0.1215	0.1215	−38.99	−0.0424	67	37
Increased absolute liver weight[c]	**F**	**Polynomial**	**0.5452**	**0.9475**	**0.9475**	**−40.00**	**0.0138**	**58**	**34**
Increased relative liver weight	M	Exponential (M2)	0.5781	0.0203	0.1161	−59.99	0.0283	55	41
Increased relative liver weight	F	Polynomial	0.7372	0.336	0.336	−37.69	0.005	59	53
Decreased epididymis weight	M	Polynomial	0.6799	0.3803	0.3803	108.84	−0.006	60	51
Increased relative spleen weight	F	Polynomial	0.4153	0.1272	0.1272	−107.17	0.016	57	34

[a]BMD modeling of data from the newborn rat study (Takahashi et al., 2004).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Selected model.

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; F = female(s); M = male(s); S-D = Sprague-Dawley.

Table C-2. Summary of BMD Modeling of Data from Young S-D Rats Treated with Picric Acid (CASRN 88-89-1) via Gavage for 28 Days[a]

Endpoint	Sex	Model	*p*-Value[b]	*p*-Value Test 2	*p*-Value Test 3	AIC for Fitted Model	Scaled Residual	BMD (mg/kg-d)	BMDL (mg/kg-d)
Increased WBC	M	Exponential (M4)	0.9489	0.0346	0.9449	173.82	0.059	3.2	1.2
Increased WBC	F	Exponential (M2)	0.5816	0.2119	0.2119	179.91	−0.552	12	9.1
Decreased RBC	M	No fit							
Decreased RBC	F	Polynomial	0.9163	0.176	0.176	197.65	−0.002	16	6.6
Decreased Hb	M	Polynomial	0.2588	0.1937	0.1937	1.7196	−0.008	21	19
Decreased Hb	F	Polynomial	0.8906	0.4384	0.4384	−8.646	−0.002	15	14
Increased MCV	F	No fit							
Decreased MCHC	F	Linear	0.4844	0.0987	0.4539	3.53	0.132	7.7	6.0
Increased Ret	M	No fit							
Increased Ret	F	No fit							
Increased absolute liver weight	M	Exponential (M2)	0.9313	0.3651	0.3651	39.03	0.001	25	15
Increased absolute liver weight	F	Polynomial	0.9379	0.2233	0.2233	30.23	−0.002	17	14
Increased relative liver weight	M	Polynomial	0.7402	0.6843	0.6843	−41.03	0.0009	24	15
Increased relative liver weight	F	Exponential (M2)	0.609	0.1685	0.1685	−53.23	−0.358	10	8.7
Increased absolute spleen weight[c]	**M**	**Exponential (M4)**	**0.1039**	**0.0739**	**0.1329**	**−66.23**	**−0.074**	**4.8**	**1.8**
Increased absolute spleen weight	F	Polynomial	0.2851	0.227	0.227	−89.53	−0.445	10	9.2
Increased relative spleen weight	M	Exponential (M2)	0.2249	0.589	0.589	−147.5	0.2107	7.1	5.6
Increased relative spleen weight	F	No fit							

Table C-2. Summary of BMD Modeling of Data from Young S-D Rats Treated with Picric Acid (CASRN 88-89-1) via Gavage for 28 Days[a]

Endpoint	Sex	Model	*p*-Value[b]	*p*-Value Test 2	*p*-Value Test 3	AIC for Fitted Model	Scaled Residual	BMD (mg/kg-d)	BMDL (mg/kg-d)
Decreased absolute epididymis weight	M	Linear	0.6673	0.5124	0.5124	−98.33	0.289	11	7.5
Decreased relative epididymis weight	M	Exponential (M2)	0.6783	0.0988	0.1138	−158.48	−0.5923	8.6	4.9

[a]BMD modeling of data from the young rat study (Takahashi et al., 2004).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Selected model.

AIC = Akaike's information criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; F = female(s); Hb = hemoglobin levels; M = male(s); MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell (erythrocyte); Ret = reticulocyte; S-D = Sprague-Dawley; WBC = white blood cell (leukocyte).

For increased absolute spleen weight in young male Sprague-Dawley (S-D) rats, with nonconstant variance model applied, all models except the Exponential Model 5 and Hill model provided an adequate fit to the variance and the means. Among all models providing adequate fit, the BMDLs estimated from different models varied greater than threefold. Therefore, $BMDL_{1SD}$ (human equivalent dose [HED]) of 1.8 mg/kg-day from the Exponential 4 model was selected.

Table C-3. Modeling Results for Increased Absolute Spleen Weight in Young Male S-D Rats Treated with Picric Acid (CASRN 88-89-1) via Gavage for 28 Days[a]

Model	Variance *p*-Value[b]	Goodness-of-Fit *p*-Value[b]	Scaled Residuals[c]	AIC	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)
Constant variance						
Exponential (Model 2)[d]	0.1329	0.1905	0.578	−67.56478	8.84	6.00
Exponential (Model 3)[d]	0.1329	0.1905	0.578	−67.56478	8.84	6.00
Exponential (Model 4)[df]	**0.1329**	**0.1039**	**−0.07437**	**−66.25554**	**4.76**	**1.80**
Exponential (Model 5)[d]	0.1329	NA	−0.5917	−65.63357	5.19	2.32
Hill[d]	0.1329	NA	−0.592	−65.633572	5.15	NA
Linear[e]	0.1329	0.2203	0.419	−67.859042	7.41	4.71
Polynomial (2-degree)[e]	0.1329	0.2203	0.419	−67.859042	7.41	4.71
Polynomial (3-degree)[e]	0.1329	0.2203	0.419	−67.859042	7.41	4.71
Power[d]	0.1329	0.2203	0.419	−67.859042	7.41	4.71

[a]Takahashi et al. (2004).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals for dose group near the BMD.
[d]Power restricted to ≥1.
[e]Coefficients restricted to be negative.
[f]Selected model.

AIC = Akaike's information criterion; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not applicable; S-D = Sprague-Dawley; SD = standard deviation.

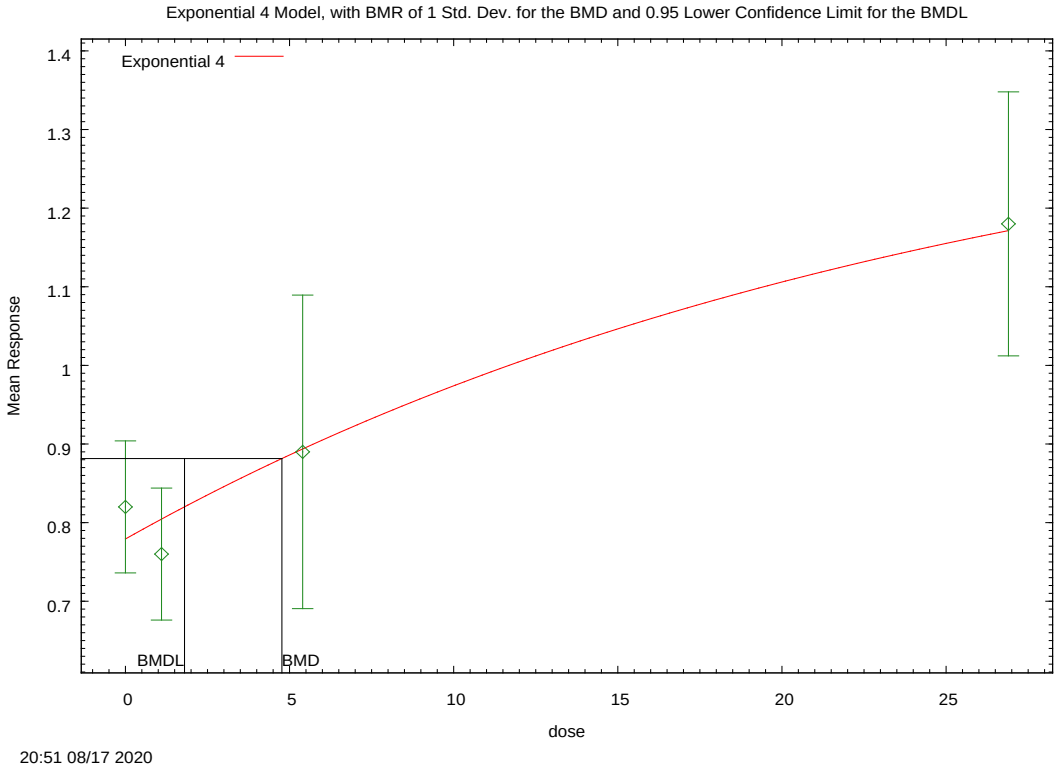


Figure C-1. BMD Output of Exponential (M4) Model for Increased Absolute Spleen Weight in Young Male S-D Rats Treated with Picric Acid (CASRN 88-89-1) via Gavage for 28 Days (Takahashi et al., 2004)

BMD Output for Figure C-1:

```
================================================================
      Exponential Model. (Version: 1.11;  Date: 03/14/2017)
      Input Data File: C:/Users/jzhao/OneDrive - Environmental Protection Agency
(EPA)/Profile/Documents/new working files/PPRTV/2019/ammonium
picrate/clearance/Phillip
review/exp_abs_spleen_wt_male_Exp-ModelVariance-BMR1Std-Up.(d)
      Gnuplot Plotting File:
                           Tue Aug 18 17:35:17 2020
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
```

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 4
lnalpha	-3.92971
rho	3.23346
a	0.722
b	0.0803637
c	1.71607
d	1 Specified

Parameter Estimates

Variable	Model 4	Std. Err.
lnalpha	-3.89679	0.393798
rho	2.65484	2.41999
a	0.779191	0.0325225
b	0.0389032	0.059696
c	1.77578	0.714234

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	6	0.82	0.08
1.1	6	0.76	0.08
5.4	6	0.89	0.19
26.9	6	1.18	0.16

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	0.7792	0.1023	0.9769
1.1	0.8045	0.1068	-1.021
5.4	0.8937	0.1228	-0.07437
26.9	1.171	0.1758	0.1198

Other models for which likelihoods are calculated:

```
  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2
```

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	37.98686	5	-65.97373
A2	41.45648	8	-66.91296
A3	39.43834	6	-66.87668
R	26.18775	2	-48.37551
4	38.11594	5	-66.23187

Additive constant for all log-likelihoods = -22.05. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 6a: Does Model 4 fit the data? (A3 vs 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	30.54	6	< 0.0001
Test 2	6.939	3	0.07386
Test 3	4.036	2	0.1329
Test 6a	2.645	1	0.1039

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

```
Specified Effect = 1.000000

       Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

             BMD =      4.76728

            BMDL =      1.79969

            BMDU =      11.8933
```

APPENDIX D. REFERENCES

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