

U.S. Army Center for Health Promotion
and Preventive Medicine

**Wildlife Toxicity Assessment for
PICRIC ACID
(2,4,6-Trinitrophenol)**

AUGUST 2005

**Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program**

**USACHPPM Document No: 37-EJ1138-01J
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Acknowledgements

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for Picric Acid (2,4,6-Trinitrophenol)

CAS Nos. 88-89-1

August 2005

1. INTRODUCTION

Picric acid (2,4,6-trinitrophenol) has been used extensively as a high explosive for military applications and more recently as a component of rocket fuel (HSDB, 2000). Nonmilitary uses of the compound include as a sensitizer in photographic emulsions, as a chemical intermediate in the production of picramic acid and chloropicrin, and as a component in matches. The ammonium salt of picric acid is used in boosters, fuses and armor-piercing shells. Given this widespread application, both now and in the past, the compound has probably been released to the environment during the manufacture of explosives and in load, assembly and pack activities at U.S. Army ammunition plants and other military installations. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of picric acid on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of the compound. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for all wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched August 16, 2000 using DIALOG to identify reviews and primary reports of studies on the toxicology of picric acid. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. In general, a two tiered approach was used in which, for laboratory mammals, all citations were first evaluated as titles and "key words in context." However, since so few hits were obtained for picric acid combined with (1) avian receptors, (2) wild mammals and (3) amphibians and reptiles (in combination), all hits for these latter receptor groups were downloaded directly as titles and abstracts. For picric acid linked to laboratory animals, 10 out of 440

titles and "key words in context" were combined with 74 titles and abstracts on wild mammals, 12 on amphibians and reptiles combined and 24 on birds to complete the tier 1 evaluation. Out of all these abstracts and titles, eight reports were marked for retrieval in tier 2, a disparity arising because the initial sweep had captured a substantial number of reports of studies that featured the use of picric acid as an immunosensitizing agent in biochemical studies. These were eliminated in tier 2 of the selection process. Details of the search strategy and the results of the search are documented in Appendix A.

In addition to DIALOG searching, the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000) was used as a secondary source.

2.2 Environmental Fate and Transport

As summarized in HSDB (2000), the widespread use of picric acid in explosives and in other military applications has likely resulted in the compound's release to the environment in various waste streams. However, no accounts of the amount of picric acid to be found in these waste streams were located in the literature. Certain aspects of the compound's likely disposition in the environment can be inferred from the compound's physico-chemical characteristics. Thus, the low vapor pressure of 7.5×10^{-7} mm Hg at 25°C suggest that picric acid may exist in both vapor and particulate phases in the ambient atmosphere (Table 1).

Table 1. Summary of Physical-Chemical Properties of Picric acid

CAS No.	88-89-1
Synonyms	2,4,6-trinitrophenol, 2-hydroxy-1,3,5-trinitrobenzene, melinite, picral, carbazotic acid
Molecular weight	229.11
Color	yellow
State	crystals
Melting point	122-123°C
Boiling point	explodes above 300°C
Odor	no odor
Solubility	Water - 12.82 g/L at 20-25 °C: soluble in benzene, chloroform, ether, ethanol, and acetone
Partition coefficients	
Log K _{ow}	1.33
K _{oc}	130 (estimate)
Vapor pressure (at 25°C)	7.5×10^{-7} mm Hg
Henry's Law constant (at 25°C)	1.7×10^{-8} atm m ³ /mole
Conversion factors	1 ppm = 9.37 mg/m ³ 1 mg/m ³ = 0.107 ppm

Sources: HSDB (2000)

Atmospheric products of photolysis are possible since picric acid absorbs light at wavelengths greater than 290 nm. Soil-borne picric acid might be expected to have high mobility, because of the compound's high solubility in aqueous solution and an estimated K_{oc} of 130. In fact, picric acid's low pKa of 0.35 also indicates that the compound will probably exist in the anionic form in soil, and, as such, display relatively limited mobility. With a Henry's Law constant of 1.7×10^{-8} atmos-cu m/mole, the compound's capacity to volatilize from moist soil surfaces is unlikely to be an important fate process. Among biotic processes, cultures of anaerobic Pseudomonads, such as *P. aeruginosa*, can transform the compound to the degradation product, 2-amino-4,6-dinitrophenol (HSDB 2000).

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Toxicity – Acute

There are very few data on the toxicity of picric acid in experimental studies, and the only studies that were found addressed the acute lethality. Thus, LD50 values of 290 ± 57.5 and 200 ± 42.9 mg/kg were determined for male and female F344 rats receiving the compound via gavage (Wyman et al., 1992). No rats died of exposures ≤ 100 mg/kg. The authors noted the steep dose mortality curve and suggested that death had probably been caused by saturation of the buffering capacity of the blood at the higher picric acid doses. Other Lowest Lethal Doses (LDLo) values have been reported (RTECS 2001), however, the value of those data could not be evaluated (Table 2.).

2.3.1.2 Mammalian Toxicity – Subacute

No data are available.

2.3.1.3 Mammalian Toxicity – Subchronic

No data are available.

2.3.1.4 Mammalian Toxicity – Chronic

van Esch et al. (1957) exposed groups of 10 Wistar rats of each sex to 500 ppm of either picric acid, hexanitrodiphenylamine, or picramide in the feed for up to 2.5 years. The purpose of this study was to evaluate the carcinogenicity of hexanitrodiphenylamine. Since the products of hydrolysis were picric acid and picramide, they were included in parallel. Nineteen control animals were used. No statistical differences in tumors were found between male or female rats; however, incidence of mammary tumors in females was high in the controls. No any other adverse effects reported. Assuming an average daily food

consumption of 0.028 kg/d and an average weight of 0.35 kg, the average daily oral exposure would be approximately 40 mg/kg-d.

2.3.1.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

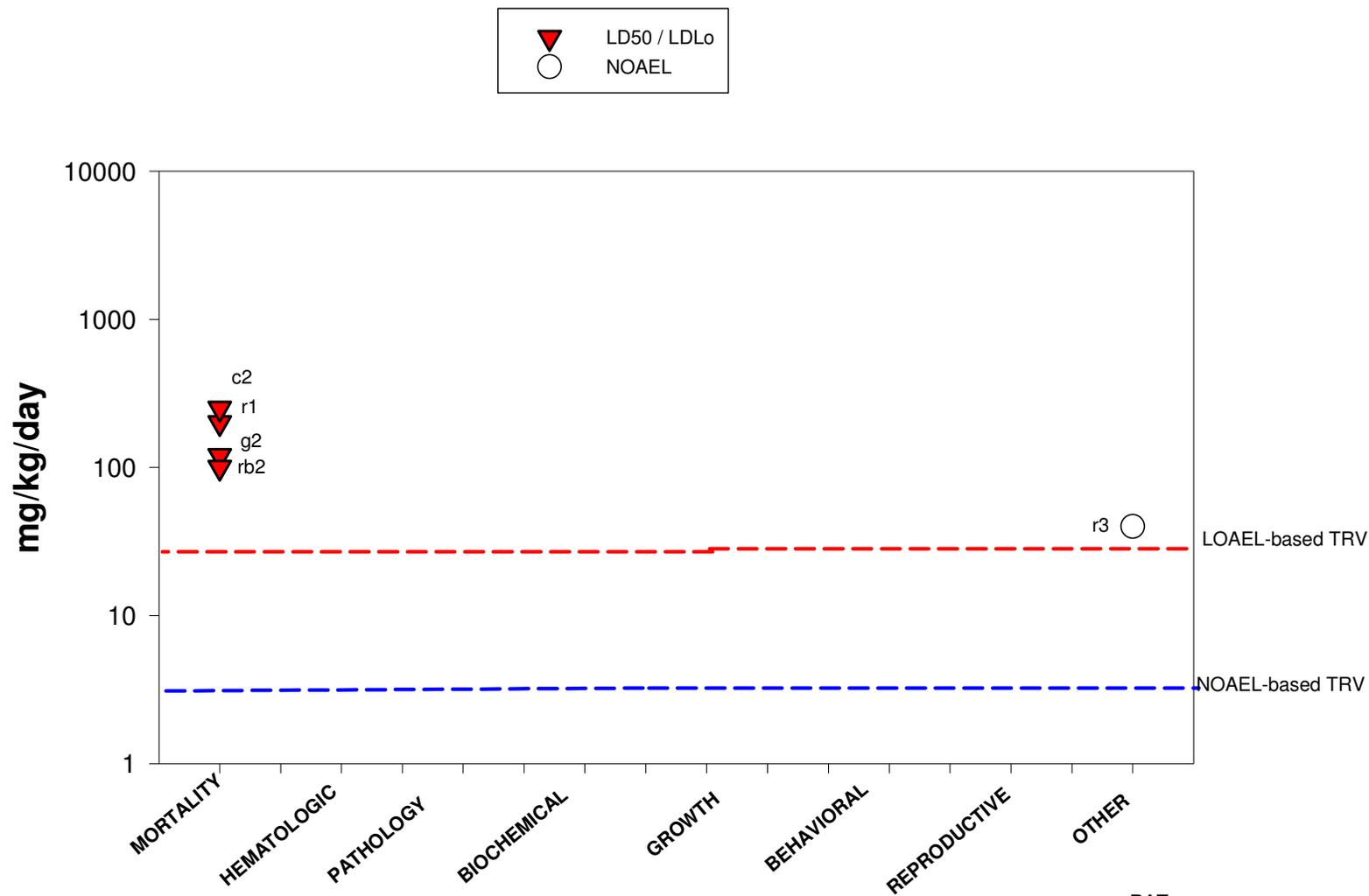
Only acute studies and a single chronic were available that evaluated the effects of oral picric acid exposure to mammals. Details are presented below (Table 2.).

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration/ type	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
Wyman et al. (1992)	Rat (F344)	LD50		200 (f) 290 (m)	Tremors, convulsions, chromodactorea, death (resulting from acidosis).
RTECS (2001) ¹	Rabbit	LDLo		120	Convulsions, diarrhea, body temperature increase.
RTECS (2001) ¹	Guinea Pig	LDLo		100	ND
RTECS (2001) ¹	Cat	LDLo		250	ND
van Esch et al. 1957	Rat (Wistar)	2.5 yrs	40	ND	No adverse effects reported.

¹ Studies are reported from a secondary sources, thus the conditions/quality of the study and accuracy of the data could not be evaluated and as such are used only to support other more qualified data.

PICRIC ACID HEALTH EFFECTS IN MAMMALS



1 = Wyman et al. 1992
 2 = RTECS 2001
 3 = van Esch et al. 1957

HEALTH EFFECTS

RAT = r
 MOUSE = m
 RABBIT = rb
 GUINEA PIG = g
 CAT = c

2.3.2 Mammalian Oral Toxicity – Other

No other data relevant to oral exposures for mammals were found.

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found.

2.3.4 Mammalian Dermal Toxicity

A single Lowest Lethal Dose (LDLo) study was reported for dogs resulting from a subcutaneous injection of 60 mg/kg (RTECS 2001). Respiratory depression along with other non-specified respiratory changes were reported. No other dermal studies conducted using animals were found.

2.4 Summary of Avian Toxicology

A single reported Lowest Lethal Dose (LDLo) was reported for pigeons resulting from a single subcutaneous injection of 200 mg/kg (RTECS 2001). No other data were available.

2.5 Amphibian Toxicology

No data are available.

2.6 Reptilian Toxicology

No data are available.

3. Recommended Toxicity Reference Values

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for ingestion exposures for the Class Mammalia

Few data were found that were conducted with picric acid. Acute data included four species from at least two orders. Results from only one repeated dosing study were found, yet the original reference could not be located. These results report no adverse effects to rats exposed to approximately 40 mg/kg-d for over 2 years (van Esch et al. 1957). Since the methods and the results from study was inadequately reported and focused primarily on the effects of hexanitrodiphenylamine, important aspects of the study (e.g., study quality) could not be addressed; therefore these data could not be used to develop a TRV for mammals.

Wyman et al. (1992) found LD50 values to be 290 and 200 for male and female rats, respectively. Blood gas analysis suggests severe acidosis as cause of death from these acute exposures. Since data are few, the approximation method must be used. The approximation method requires that uncertainty

factors (100 to the female LD50 for the NOAEL-based TRV and 20 for the LOAEL-based TRV) be applied to these acute data (USACHPPM 2000). Therefore, a mammalian NOAEL-based TRV of 2 mg/kg-d and a LOAEL-based TRV of 10 mg/kg-d. These values are in the same magnitude of the NOAEL reported by van Esch et al. (1957). Both are given a LOW degree of confidence based on the paucity of the data for picric acid. Given the chemical structural similarities between picric acid and other nitroaromatics (e.g., 2,4,6-trinitrotoluene, 1,3,5-trinitrobenzene), these values are within similar ranges and seem reasonable.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	2 mg/kg/d	Low
LOAEL-based	10 mg/kg-d	Low

3.1.1.1 TRVs for ingestion exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, these data are representative of omnivorous rodents, and may be considered as such. More data for other species would be needed to derive values for other foraging guilds.

3.1.2 TRVs for inhalation exposures for the Class Mammalia

Not available at this time.

3.1.3 TRVs for dermal exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Sufficient data are not available to derive a TRV for birds at this time.

4. Important Research Needs

Few data for animals exist for picric acid at this time. Long-term repeated dosing studies are needed for mammals as well as other vertebrates to develop useful TRV values. However, chemical structural similarities between picric acid and other nitroaromatics may make using surrogates useful in the interim.

5. References

HSDB (Hazardous Substances Databank). 2000. On-line Database. National Library of Medicine. Washington, DC.

RTECS (Registry of Toxic Effects of Chemical Substances). 2001. On-line Database. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services. Washington, DC.

USACHPPM (U.S. Army Center for Health Promotion and Preventive Medicine). 2000. *Standard Practice for Wildlife Toxicity Reference Values*, Technical guide 254.

van Esch, G.T., H.H. Vink, and H. van Genderen. 1957. Influence of hexanitrodiphenylamine on the incidence of neoplasms in the mammary tissue of rats. *Nature*. 5484 (180): 509-510.

Wyman, J.F., M.P. Serve, D.W. Hobson, L.H. Lee and D.E. Uddin. 1992. Acute toxicity, distribution, and metabolism of 2,4,6-trinitrophenol (picric acid) in Fischer 344 rats. *J. Toxicol. Environ. Health* 37: 313-327.

APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONMENTAL, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- ◆ RD (reduce duplicates)

The search strategy for **Birds**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- ◆ RD

The search strategy for **Laboratory Mammals**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- ◆ NOT ((meeting()poster) or (meeting()abstract))
- ◆ NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- ◆ RD

The search strategy for **Wild Mammals**:

- ◆ Chemical name, synonyms, CAS numbers
- ◆ And(didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or myocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search.

As described in Section 2.1, 440 "key words in context" and titles of articles linking laboratory mammals with picric acid were evaluated along with 110 titles and abstracts linking picric acid to birds, reptiles, amphibians and wild mammals. Eight articles were chosen for retrieval from these tier 1 items in tier 2 of the selection process.

etrieved for this survey.