FINAL
9-15-2009

Provisional Peer-Reviewed Toxicity Values for

Picramic Acid
(CASRN 96-91-3)

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

COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR PICRAMIC ACID (CASRN 96-91-3)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Picramic acid, or 4,6-dinitro-2-aminophenol, is used as a chemical intermediate in the manufacture of azo dyes, an explosive initiator, an oxidation base for dyeing furs, a reagent for albumin, and an acid-base indicator (Hazardous Substances Data Bank [HSDB], 2008). Figure 1 shows its chemical structure.

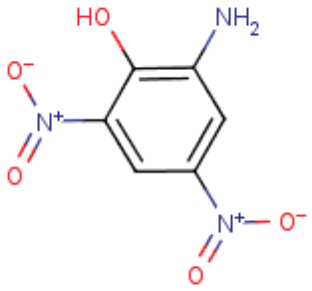

Figure 1. Structure of Picramic Acid

There is no assessment of picramic acid (2-amino-4,6-dinitrophenol) on IRIS (U.S. EPA, 2008), in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) or in the Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994) includes Health and Environmental Effects Profiles (HEEPs) for aminophenols (U.S. EPA, 1985) and selected dinitrophenols (U.S. EPA, 1984); however, neither of these include data on picramic acid. Similarly, the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) prepared a toxicological profile of dinitrophenols, but this document did not include picramic acid. Occupational exposure limits for picramic acid have not been derived by the American Conference for Governmental Industrial Hygienists (ACGIH, 2008), the National Institute for Occupational Safety and Health (NIOSH, 2008), or the Occupational Safety and Health

Administration (OSHA, 2008). Assessments for picramic acid have not been performed by the National Toxicology Program (NTP, 2005, 2008), the California Environmental Protection Agency (CalEPA, 2008a,b), the International Agency for Research on Cancer (IARC, 2008), or the World Health Organization (WHO, 2008).

Literature searches were conducted from 1960s through November 2008 for studies relevant to the derivation of provisional toxicity values for picramic acid. Databases searched include MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months). A Cosmetic Ingredient Review Expert Panel Report (CIREP, 1992) was also consulted for relevant information.

REVIEW OF PERTINENT DATA

Human Studies

No relevant data regarding the toxicity of picramic acid to humans following chronic or subchronic oral or inhalation exposure were located.

Animal Studies

No relevant data regarding the toxicity of picramic acid to animals following chronic or subchronic oral or inhalation exposure were located.

Other Studies

Acute Toxicity

The acute oral LD_{50} for picramic acid in male HA/ICR mice was 378 mg/kg (CIREP, 1992). Few details of the study are reported in the CIREP (1992) review article: only that 36 adult male mice were tested using a wide range of doses, and that the LD_{50} was determined by probit analysis.

Robbins (1944) reported that picramic acid in the diet of chicks at a concentration of 1% resulted in lesions (lens opacities or cataracts) similar to those observed with 2,4-dinitrophenol at a concentration of 0.25%. No further information was provided on the experiments conducted with picramic acid. In the experiments with 2,4-dinitrophenol, the compound was mixed with feed and given ad libitum to chicks (0.05−0.25% in feed) and ducks (0.25% in feed) for durations ranging from 3 to 31 days. Each day, the fowl were examined for lens opacities and their temperatures and body weights were recorded. Individual birds were sacrificed at intervals from 5 hours to 30 days after commencement of exposure, and their lenses were examined microscopically. Lens opacities were observed within hours of the initial administration of 2,4-dinitrophenol at a dietary concentration of 0.25%.

Other Routes

In a brief report published in French in 1933, Casier (1933) reported that sodium picramate exposure caused hyperthermia, sometimes resulting in death, when administered intravenously to dogs or via intravenous or intraperitoneal injection to pigeons. The author indicated that the hyperthermic effects were similar to those occurring with dinitronaphthol, dinitrophenol, or dinitrocresol, but were less severe and lasted longer.

Pugh and Stone (1968) measured bile secretion, bromosulphophthalein excretion, and body temperature in anaesthetized male and female dogs (strain and number not reported) exposed via intravenous injection to doses of 5−53 mg/kg picramic acid. Picramic acid exposure resulted in a moderate increase in bile flow (mean 97% increase in bile volume relative to controls) and a slight decrease in bromosulphophthalein excretion (2% less than controls), but it did not affect body temperature. By comparison, 2,4-dinitrophenol at a dose of 5 mg/kg caused a larger increase in bile volume (153% of controls), an increase in bromosulphophthalein excretion (16% higher than controls), and an increase in body temperature (3.9°C).

The effects of dermal exposure to oxidative hair dyes containing picramic acid or sodium picramate (at concentrations from 0.01 to 0.1%) were assessed in subchronic, chronic, and teratogenicity studies. The dye was mixed with an equal volume of 6% hydrogen peroxide and applied twice weekly at a dose of 1 mL/kg. No effects on hematology, blood chemistry, urinalysis, or gross or microscopic findings were observed in New Zealand white rabbits (6/sex) exposed dermally to a hair dye containing 0.1% sodium picramate for 13 weeks; slight thickening of the skin was noted at the application site (Burnett et al., 1976). Teratogenicity testing of the same dye (with 0.1% sodium picramate) applied to the shaven skin of 20 mated female CD rats on gestation days 1, 4, 7, 10, 13, 16, and 19 (at 2 mL/kg of the dye solution) revealed no effects on numbers of corpora lutea, implantation sites, live fetuses, resorption sites, or the incidence of visceral or skeletal malformations (Burnett et al., 1976). The carcinogenicity of an oxidative hair dye containing 0.01% picramic acid was evaluated in a skin-painting study using Swiss-Webster mice (Jacobs et al., 1984, as cited in CIREP, 1992). The dye was mixed with an equal volume of hydrogen peroxide and applied to the skin once a week for 20 months. Treatment caused no hematology or urinalysis changes, and did not increase the incidence of tumors compared with controls (Jacobs et al., 1984, as cited in CIREP, 1992). Citing unpublished studies, CIREP (1992) reported that an aqueous solution of 2.5% picramic acid did not cause skin irritation in three rabbits (strain not reported), but a solution of 2% picramic acid (1:10, 1:100, and 1:1000 dilutions in distilled water) caused mild skin sensitization in guinea pigs (4/15 guinea pigs treated with the 1:10 dilution of 2% picramic acid), and an aqueous solution of 2.5% sodium picramate caused “mild conjunctival inflammation” as ocular irritation in three albino rabbits.

Toxicokinetics

No information on the pharmacokinetics of picramic acid in humans or animals was located in the available literature either via oral or inhalation route. Wyman et al. (1992) reported that the primary urinary metabolite of picric acid administered orally to rats was picramic acid, which represented 18.5% of the administered dose. Picramic acid was one of two urinary metabolites (along with picric acid) observed in a study of rats exposed subcutaneously to trinitrophenylmethylnitramine (Myers and Spinnato, 2007). The study did not quantify the excretion of either picric acid or picramic acid. A similar metabolism of trinitrophenylmethylnitramine (Tetryl) to picric acid and picramic acid was also observed in a rabbit (strain not stated) oral study by Zambrano and Mandovano (1956). The exact quantification of the excretion of picric acid and picramic acid was not provided, but the picramic acid was present in the urine of “practically all the rabbits treated.” The study authors also stated that picramic acid can be excreted in a conjugated form (sulfoconjugates).

Genotoxicity

With or without metabolic activation, picramic acid yielded positive results for reverse mutation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537, and TA1538 (Zeiger et al., 1988; Litton Bionetics, Inc., 1979a, as cited in CIREP, 1992; Wyman et al., 1979; CIREP, 1992; Zieger et al. 1988) but gave equivocal results in *Saccharomyces cerevisiae* strain D4 (CIREP, 1992). Picramic acid was not mutagenic at the TK locus in cultured L5178Y mouse lymphoma cells at concentrations that were moderately toxic, although there was some evidence of weak mutagenic activity with extremely toxic treatments (2−4% relative growth). This compound did not increase the frequency of sister chromatid exchanges (SCE) in L5178Y mouse lymphoma cells (CIREP, 1992). Picramic acid did not induce chromosomal aberrations in the bone marrow of mice in a subchronic group given oral doses up to 75.6 mg/kg (CIREP, 1992). In addition, picramic acid gave negative results in a dominant lethal assay using CD-1 mice (CIREP, 1992).

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR PICRAMIC ACID

Due to a lack of data, no chronic or subchronic RfDs are developed. However, the Appendix of this document contains a Screening Value (a screening RfD) based on an analog treatment, which may be useful in certain instances. Please see the attached Appendix for details.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR PICRAMIC ACID

There are no toxicity data from which to derive a provisional inhalation RfC (p-RfC) for picramic acid. Furthermore, there are no inhalation toxicity data for any of the structural analogs identified for picramic acid, precluding derivation of a chronic screening RfC.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR PICRAMIC ACID

Weight-of-Evidence Descriptor

Under the 2005 *Guidelines for Carcinogen Assessment* (U.S. EPA, 2005), there is "*Inadequate Information to Assess* [the] *Carcinogenic Potential*" of picramic acid. There are no data with which to assess the potential carcinogenicity of picramic acid in humans or animals. Genotoxicity testing of picramic acid yielded positive results for mutagenicity in *S. typhimurium*, equivocal results for mutagenicity in *S. cerevisiae*, negative results for mutagenicity and SCE induction in mouse lymphoma cells, and negative results for chromosomal aberration induction in mice.

Quantitative Estimates of Carcinogenic Risk

The lack of data on the carcinogenicity of picramic acid precludes the derivation of quantitative estimates of risk for either oral (p-OSF) or inhalation (p-IUR) exposure.

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APPENDIX. DERIVATION OF A SCREENING VALUE FOR PICRAMIC ACID

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for picramic acid. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center 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 PPRTV documents 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 is considerably 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 Superfund Health Risk Technical Support Center.

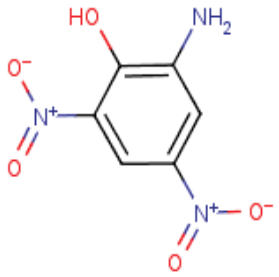
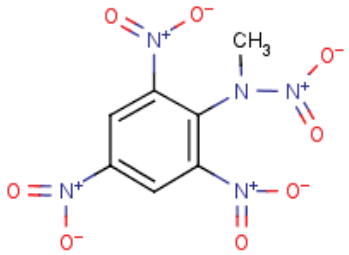
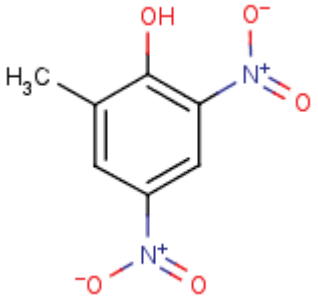
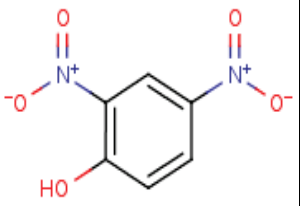
Identification of Structural Analogs

Only compounds that have toxicity values available on IRIS, the current PPRTV status table, or the HEAST (U.S. EPA, 1997) were considered as potential structural analogs to picramic acid. The ChemIDplus Similarity Search function was used both as a tool to identify candidate analogs and as a quantitative measure of structural similarity by which to select the final analog to represent the subject chemical. The approach used to select potential analogs consisted of two steps:

1) Using available toxicokinetic information, upstream and/or downstream metabolites of picramic acid in mammalian systems were identified and considered as potential analogs if they had toxicity data from the pertinent sources (IRIS, PPRTV, HEAST).
2) Using the ChemIDplus Similarity Search function, compounds structurally similar to picramic acid were identified. Beginning with the highest degree of similarity, compounds with structural similarity scores of at least 85% were identified, and IRIS, the current PPRTV status table, and the HEAST were searched for the CAS registry numbers and names of these compounds. The similarity score was later relaxed to at least 60% to include any potential analogs.

Applying this approach, three potential analogs were identified for picramic acid. The first candidate analog, trinitrophenylmethylnitramine (also known as tetryl or nitramine), was identified because picramic acid is a metabolite of this compound. Picramic acid was one of two urinary metabolites (along with picric acid) observed in a study in rats exposed subcutaneously to trinitrophenylmethylnitramine (Myers and Spinnato, 2007). While picramic acid is also a metabolite of picric acid (Wyman et al., 1992), picric acid does not have a toxicity value available from IRIS, the PPRTV database, or the HEAST. The search of structural analogs by the ChemIDplus similarity score identified trinitrophenylmethylnitramine (Tetryl), 4,6-dinitro-o-cresol (DNOC), and 2,4-dinitrophenol (2,4-DNP) as candidate analogs (see Tables A-1 and A-2).

Table A-1. Physical-Chemical Properties of Picramic Acid and Candidate Analogs[a]

	Picramic Acid (4,6-Dinitro-2-aminophenol)	Trinitrophenylmethylnitramine (Tetryl or Nitramine)	4,6-Dinitro-o-cresol (DNOC)	2,4 Dinitrophenol (2,4-DNP)
Structure				
CASRN	96-91-3	479-45-8	534-52-1	51-28-5
Molecular formula	C6-H5-N3-O5	C7-H5-N5-O8	C7-H6-N2-O5	$CH_4N_2O_5$
Molecular weight	199.122	287.15	198.133	184.11
ChemID Plus Similarity Score (%)	100	62.68	88.59	85.47
Melting point (°C)	169	131.5	86.6	115.5
Boiling point (°C)	-	-	378	-
Vapor Pressure (mm Hg at 25°C)	4.16×10^{-7}	5.66×10^{-8}	1.06×10^{-4}	3.9×10^{-4} at 20°C
Henry's Law Constant (atm-m^3/mole at 25°C)	9.75×10^{-12}	2.71×10^{-9}	1.4×10^{-6}	8.6×10^{-8} at 20°C
Water solubility (mg/L)	1400 at 22°C	74	198 at 20°C	2790 at 20°C
Log K_{ow}	0.93	1.64	2.12	1.67
pKa	1	-	4.31	4.09 at 25°C

[a]Data reported by ChemIDplus

Table A-2. Comparison of Available Toxicity Data for Picramic Acid and Candidate Analogs[a]

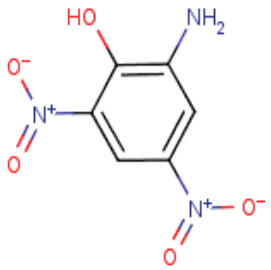
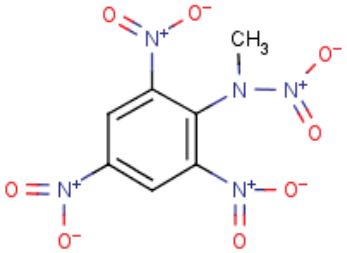
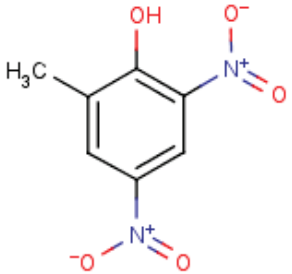
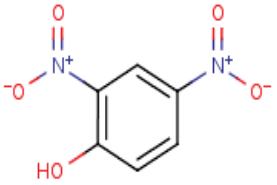
	Picramic Acid (4,6-Dinitro-2-aminophenol)	**Trinitrophenylmethylnitramine (Tetryl or Nitramine)**	**4,6-Dinitro-o-cresol (DNOC)**	**2,4 Dinitrophenol (2,4-DNP)**
Structure				
CASRN	96-91-3	479-45-8	534-52-1	51-28-5
ChemID Plus Similarity Score (%)	100	62.68	88.59	85.47
Human LD_{LO} or LC_{LO}	-	-	29 mg/kg (unreported route); 500 mg/kg (dermal)	36 mg/kg (oral)
Human TD_{LO} or TC_{LO} (Route, Effect)	-	-	7.5 mg/kg (oral; sleepiness, headache) 1 mg/m^3 (inhalation; CNS effects, cardiac and gastrointestinal changes)	-
Oral LD_{50} in Mice (mg/kg)	378[b]	5000 (LD_{LO})	21	45
Intravenous LD_{50} in Dogs (mg/kg)	150 (LD_{LO})	-	15 (LD_{LO})	15 (LD_{LO})
Subcutaneous LD_{50} in Rat (mg/kg)	2100 (LD_{LO})	-	25.6	25

Table A-2. Comparison of Available Toxicity Data for Picramic Acid and Candidate Analogs[a]

	Picramic Acid (4,6-Dinitro-2-aminophenol)	Trinitrophenylmethylnitramine (Tetryl or Nitramine)	4,6-Dinitro-o-cresol (DNOC)	2,4 Dinitrophenol (2,4-DNP)
Chronic Oral RfD (mg/kg-day) Critical Effect Source	-	4×10^{-3} Methemoglobin and serum chemistry changes indicative of hepatotoxicity in rats U.S. EPA, 2004 (PPRTV)	1×10^{-4} Discolored conjunctivae, elevated body temperature and basal metabolic rate, perspiration, fatigue in humans U.S. EPA, 2002 (PPRTV) 1×10^{-4} Cataracts in humans Derived in this Appendix with a LOAEL of 1.2 mg/kg-day (Måhlén, 1938) and a composite UF of 10,000	2×10^{-3} Cataracts in humans U.S. EPA, 1991b (IRIS)

[a]Data reported by ChemIDplus except where noted
[b]CIREP (1992)

Selection of Surrogate for Picramic Acid

The selection of a surrogate for picramic acid from among the three candidate analogs took into consideration the physical-chemical properties of the compounds and available information on their toxicity. The physical-chemical properties and available toxicity information on picramic acid were compared with corresponding information on the three candidate analogs in Tables A-1 and A-2 below. The comparisons among the physical-chemical properties of picramic acid and its candidate analogs suggest differences in properties that may be important determinants of the compounds' behavior in biological systems. Specifically, water solubility, Henry's Law constants, and $\log K_{ow}$ values differed widely among the analogs; consequently, these data show that surrogate selection based on the physiochemical properties alone may not be appropriate. The comparison of acute lethality information suggests that the candidate analogs are either much less acutely lethal (Tetryl) or much more acutely lethal (DNOC and 2,4-DNP). As Tetryl is less acutely lethal than picramic acid, and, therefore, could potentially underestimate the toxicity of picramic acid in the long term, this compound was not considered further as the surrogate for picramic acid. Furthermore, Tetryl was also excluded based on lack of similar critical effect(s) to picramic acid, DNOC, and 2,4-DNP (see Table A-2). There is limited direct information on potential target(s) or critical effect(s) of picramic acid toxicity. Robbins (1944) reported that picramic acid administered in the diet of chicks resulted in cataracts similar to those observed with 2,4-dinitrophenol. Therefore, it is likely that picramic acid can cause cataract formation in humans as well as in chickens (chemical similarity between picramic acid and 2,4-dinitrophenol is at least 85%; see Table A-1).

Comparison of DNOC and 2,4-DNP

A summary of the critical study is excerpted from the U.S. EPA (1991b) IRIS record for 2,4-DNP and reproduced here for the reader's convenience.

> *Over 100 anecdotal cases of cataracts resulting from therapeutic use of 2,4-dinitrophenol were reviewed. The length of time and amount of drug taken varied among the population. It was estimated that over 1% of the population administered 2,4-dinitrophenol developed cataracts. Data did not allow for calculation of a NOEL; cataracts were observed in patients receiving as little as 2 mg/kg/day, the lower range of the recommended therapeutic dose.*

Similarly, a summary of the critical studies is excerpted from the U.S. EPA (2002) PPRTV document for DNOC and reproduced here for the reader's convenience.

> *The human studies, particularly Harvey et al. (1951), Dodds and Robertson (1933), Plotz (1936) and Måhlén (1938), indicate that dosages of 0.35 to ≈1 mg/kg-day constitute a LOAEL for humans ingesting DNOC for up to 1 year. Critical effects include elevation of BMR and body temperature, excessive perspiration, fatigue, discoloration of conjunctivae, and cataract formation. The low end of the LOAEL range, 0.35 mg/kg-day (Plotz, 1936), is chosen as the basis for the calculation.*

Even though a LOAEL of 0.35 mg/kg-day (Plotz, 1936) was originally chosen as the most sensitive endpoint (not based on formation of cataracts in humans) and, therefore, as the point of departure (POD) for DNOC in the U.S. EPA (2002) PPRTV, an alternate LOAEL of 1.2 mg/kg-day based on cataracts in humans (Måhlén, 1938) is specifically selected for the derivation of a chronic screening RfD in this Appendix. Details of the Måhlén (1938) study is summarized as the follows:

Måhlén (1938) reported on 73 patients in Sweden who had received DNOC at weight-reduction dosages (≈1 mg/kg-day) during a clinical study (DNOC was taken as a dieting drug). Durations of treatment for these patients were not specified. After some time on treatment, a nonjaundice yellowing of the skin and sclera were observed; however, reduction of the dosage eliminated this symptom (reversible). Six of the patients were examined for cataracts by other physicians and were found to be negative. The remaining 67 patients were examined by the study author. Of these 67, 11 were eliminated from consideration because they had senile cataracts (not treatment-related), had other conditions such as diabetes, or, in the case of one patient, were not yet on DNOC treatment at the time of the examinations. Of the remaining 56 patients, 1 had bilateral cataracts attributable to DNOC. The affected patient was a 31-year old woman had taken 83 mg/day (1.2 mg/kg-day based on her body weight of ≈69 kg at the time of observation) of DNOC for 56 days and developed cataracts about 8 months later.

Måhlén (1938) also describes six other cases of cataracts associated with ingestion of DNOC in Sweden. These cases were identified through a questionnaire sent by the Swedish Board of Health to Swedish ophthalmologists. In all six cases, the patients were women (ages ranged from 34−57 years), and their daily doses of DNOC ranged from 50−125 mg/day. Although body weights were not specified, assuming body weights of 60 kg would result in doses of 0.8−2.1 mg/kg-day. Durations of treatment ranged from 5−12 months, with occasional additional treatment periods after 1−2 months without treatment. Symptoms and diagnosis of cataracts occurred 2−10 months after the end of treatment. Based on the specific clinical case with reported body weight (69 kg) and daily dose (83 mg/day), a LOAEL of 1.2 mg/kg-day is identified for DNOC-induced cataract development.

As a result, this alternate endpoint and its associated LOAEL are deemed more appropriate for DNOC in the limited context of serving as a potential surrogate for picramic acid.

The LOAEL of 1.2 mg/kg-day in a subchronic human study (Måhlén, 1938; U.S. EPA, 2002) for DNOC and the LOAEL of 2 mg/kg-day in a subchronic human study (Horner, 1942; U.S. EPA, 1991b) for 2,4-DNP both were based on formation of cataracts in humans as the critical effects, suggesting that cataract formation may be a common critical effect for the dinitrophenols (both DNOC and 2,4-DNP have a common core structure of dinitrophenol). Therefore, cataract formation in humans is postulated to be the critical effect for picramic acid because picramic acid belongs to this dinitrophenol chemical class.

For 2,4-DNP, U.S. EPA (1991b) derived a chronic RfD of 2×10^{-3} mg/kg-day based on the LOAEL of 2 mg/kg-day as the POD and applied a composite UF of 1,000 (see U.S. EPA, 1991b for details). The composite UF includes a UF_H of 10 for intraspecies variation, a UF_S of 10 for extrapolation from subchronic-to-chronic duration, and a UF_L of 10 for extrapolation from LOAEL to NOAEL.

Screening Chronic RfD

Although the U.S. EPA (2002) derived a p-RfD of 1×10^{-4} mg/kg-day for DNOC based on the LOAEL of 0.35 mg/kg-day and a composite UF of 3,000 (see U.S. EPA, 2002 for details), an alternate chronic p-RfD (as a potential surrogate for picramic acid), based on the LOAEL of 1.2 mg/kg-day[1] for formation of cataracts in humans (Måhlén, 1938), is derived specifically for this Appendix as follows:

Screening Chronic RfD = LOAEL ÷ UF
= 1.2 mg/kg-day ÷ 10,000
= **0.0001 or 1×10^{-4} mg/kg-day**

The composite UF of 10,000 for picramic acid (not for DNOC) is composed of the following:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because the data for evaluating susceptible human response are insufficient.
- UF_L: A factor of 10 is applied for extrapolation from LOAEL to NOAEL because the data for identifying a NOAEL are insufficient.
- UF_D: A factor of 10 is applied for database inadequacies—including lack of a multigeneration reproduction study and a developmental study.
- UF_S: A factor of 10 is applied to derive the chronic RfD because of an adjustment for exposure from subchronic-to-chronic duration.

Notably, this composite UF is based on lack of toxicity information for picramic acid (using default approach for each UF)—not for DNOC. This alternate p-RfD of 1×10^{-4} mg/kg-day is only applicable in this Appendix for comparison of common critical effect (cataract formation) across a specific chemical class (dinitrophenols), and it should not be used for DNOC by itself or elsewhere. In the future, if newer studies suggest that picramic acid could cause similar responses (i.e., headache, excessive perspiration, and fatigue, elevated BMR, body temperature, and greenish coloration of sclera) as shown for DNOC in Plotz (1936), then the critical effect and POD of picramic acid, and the composite UF could be changed accordingly (as stated in the U.S. EPA, 2002 document or the updated PPRTV for DNOC).

Both DNOC and 2,4-DNP are suitable candidates as the appropriate surrogate for picramic acid because they have the same critical effect and target organ (cataracts; eyes) and comparable acute lethality data. DNOC differs from picramic acid by only one functional group, a methyl group instead of amino group, and 2,4-DNP lacks this additional functional group. DNOC is, therefore, considered

[1] A molecular weight (MW) adjustment is only necessary when a mechanism of action is elucidated and when molecular targets have been identified. It is a common misconception that a MW adjustment must be applied indiscriminately for the use of any surrogate approach when there is no mechanistic information (down to the molecular level). This misconception stems from the practice of pharmaceuticals when one applies a structure-activitiy relationship (SAR) to a series of congeners with a common and elucidated molecular mechanism (e.g. tamoxifen vs. substituted tamoxifens binding to estrogen receptor alpha [ERα]). In general, a consideration of the MW adjustment is not necessary if the MW of potential surrogate is less than a factor of 2 in comparison to the MW of chemical of concern. If the MWs of potential surrogates are significantly higher (>2-fold) than the MW of chemical of concern, then a common mechanism of action should be elucidated prior to the molecular-weight adjustment.

structurally more similar to picramic acid than 2,4-DNP. Based on the ChemIDplus similarity score (88.59% for DNOC vs. 85.47% for 2,4-DNP; see Table A-2) and available RfDs (1×10^{-4} for DNOC vs. 2×10^{-3} for 2,4-DNP), DNOC was selected as the surrogate for picramic acid.

For picramic acid, the chronic p-RfD of 1×10^{-4} mg/kg-day, derived in this Appendix and based on formation of cataracts in a subchronic human study (Måhlén, 1938), is recommended only as a chronic screening RfD based on the surrogate analysis and structure-activity relationship presented here. This alternate chronic p-RfD (a toxicity-based exposure level that is specific to the target organ/effect of interest) uses the LOAEL of 1.2 mg/kg-day and employs a composite UF of 10,000 (10 for intraspecies extrapolation, 10 for LOAEL-to-NOAEL extrapolation, 10 for subchronic-to-chronic exposure duration extrapolation, and 10 for database inadequacies) because data for evaluating developmental/reproductive toxicity for picramic acid are inadequate.

Confidence in the critical study (low) and database (low) is the same as stated in the PPRTV for DNOC (see U.S. EPA, 2002). Confidence in the overall surrogate approach is high because the structural similarity is reasonably high (≈89%) between picramic acid and DNOC and because of the common target organ (eyes) and toxic effect (cataracts). However, due to the high inherent uncertainty in the overall surrogate approach and low confidence in the critical study and database, confidence in the screening chronic p-RfD is low.