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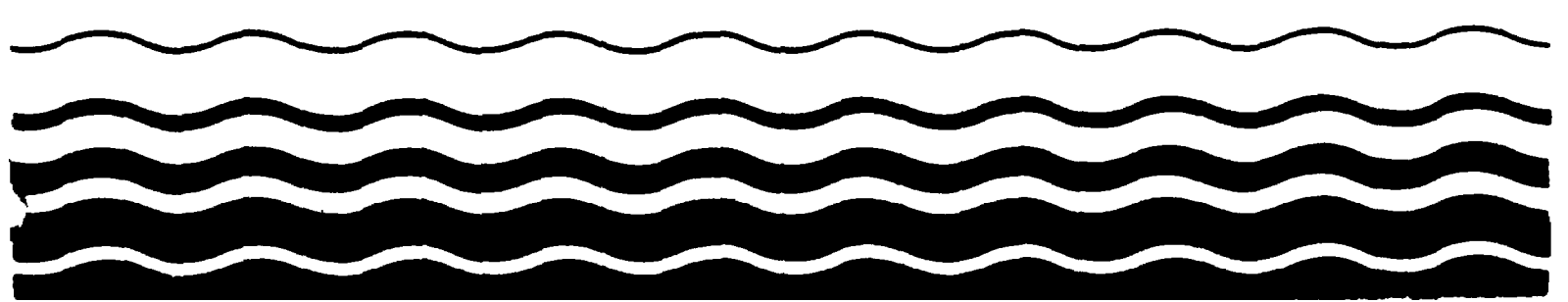
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Ambient Water Quality Criteria for Nitrophenols



AMBIENT WATER QUALITY CRITERIA FOR
NITROPHENOLS

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U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisfaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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CRITERIA DOCUMENT

NITROPHENOLS

CRITERIA

Aquatic Life

The available data for nitrophenols indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 230 ug/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive freshwater aquatic life but toxicity to one species of algae occurs at concentrations as low as 150 ug/l.

The available data for nitrophenols indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 4,850 ug/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive saltwater aquatic life.

Human Health

Due to the insufficiency in the available data for mono- and trinitrophenols, satisfactory criteria cannot be derived at this time, using the present guidelines.

For the protection of human health from the toxic properties of dinitrophenols and 2,4-dinitro-o-cresol ingested through water and contaminated aquatic organisms, the ambient water criteria are determined to be 70 ug/l and 13.4 ug/l, respectively.

For the protection of human health from the toxic properties of dinitrophenols and 2,4-dinitro-o-cresol ingested through contaminated aquatic organisms alone, the ambient water criteria are determined to be 14.3 mg/l and 755 ug/l, respectively.

INTRODUCTION

Mononitrophenol has three isomeric forms, distinguished by the position of the nitro group on the phenolic ring. Three isomeric forms are possible, namely 2-nitrophenol, 3-nitrophenol, and 4-nitrophenol. The compounds are also commonly referred to as o-nitrophenol, m-nitrophenol, and p-nitrophenol, respectively.

Commercial synthesis of 2-nitrophenol and 4-nitrophenol is accomplished through the hydrolysis of the appropriate chloronitrobenzene isomers with aqueous sodium hydroxide at elevated temperatures (Howard, et al. 1976). Production of 3-nitrophenol is achieved through the diazotization and hydrolysis of m-nitroaniline (Matsuguma, 1967). The mononitrophenol isomers are used in the United States primarily as intermediates for the production of dyes, pigments, pharmaceuticals, rubber chemicals, lumber preservatives, photographic chemicals, and pesticidal and fungicidal agents (U.S. International Trade Commission, 1976). As a result of this use pattern, the major source for environmental release of mononitrophenols is likely to be from production plants and chemical firms where the compounds are used as intermediates. The mononitrophenols may also be inadvertently produced via microbial or photodegradation of pesticides which contain mononitrophenol moieties. Approximately 10 to 15 million pounds of 2-nitrophenol are produced annually (Howard, et al. 1976) for uses including synthesis of o-aminophenol, o-nitroanisole, and other dye stuffs (Matsuguma, 1967; Howard, et al. 1976). Although production figures for 3-nitrophenol are not available, Hoecker, et al. (1977) estimate that production is less than one million pounds annually. 3-Nitrophenol is used in the manufacture of dye intermedi-

ates such as anisidine and m-aminophenol (Kouris and Northcott, 1963; Matsuguma, 1967). 4-Nitrophenol is probably the most important of the mononitrophenols in terms of quantities used and potential environmental contamination. Demand for 4-nitrophenol was 35,000,000 pounds in 1976 and production is projected to increase to 41,000,000 pounds by 1980 (Chemical Marketing Reporter, 1976). Most of the 4-nitrophenol produced (87 percent) is used in the manufacture of ethyl and methyl parathions. Other uses (13 percent) include the manufacture of dye-stuffs and n-acetyl-p-aminophenol (APAP) and leather treatments. A possible source of human exposure to 4-nitrophenol is as a result of microbial or photodegradation of the parathions. In vivo production of 4-nitrophenol following absorption of parathion or other pesticides by humans is another possible source of human exposure.

Physical and chemical properties of the mononitrophenols are summarized in Table 1.

Dinitrophenols

Six isomeric forms of dinitrophenol are possible, distinguished by the position of the nitro-groups on the phenolic ring. Of the six possible dinitrophenol isomers, 2,4-dinitrophenol is by far the most important. The most recent production figure for 2,4-dinitrophenol is 863,000 lb. reported by the U.S. International Trade Commission (1968). Approximate consumption per year is estimated at 1,000,000 lbs. (Howard, et al. 1976). 2,4-Dinitrophenol is used primarily as a chemical intermediate for the production of sulfur dyes, azo dyes, photochemicals, pest control agents, wood preservatives, and explosives (Matsuguma, 1967; Perkins, 1919; Springer, et al. 1977a,b).

TABLE 1
Properties of Mononitrophenols*

	2-Nitrophenol	3-Nitrophenol	4-Nitrophenol
Formula	$C_6H_5NO_3$	$C_6H_5NO_3$	$C_6H_5NO_3$
Molecular Weight	139.11	139.11	139.11
Melting Point (°C)	44-45	97	113-114
Boiling Point (°C)	214-216	279	279
Density	1.485	1.485	1.479
Water Solubility (g/l)	0.32 at 38°C 1.08 at 100°C	1.35 at 25°C 13.3 at 90°C	0.804 at 15°C 1.6 at 25°C
Vapor Pressure	1 mm Hg at 49.3°C		
Ka	7.5×10^{-8}	5.3×10^{-9}	7×10^{-8}

*Sources: Windholz, 1976; Weast, 1975; Matsuguma, 1967.

Production figures and usage data for the remaining five dinitrophenol isomers are not available. It is reasonable to assume that production and usage of these compounds are extremely limited in the United States.

Commercial synthesis of 2,4-dinitrophenol is accomplished by the hydrolysis of 2,4-dinitro-1-chlorobenzene with sodium hydroxide at 95 to 100°C (Matasuguma, 1967). As a result of the use pattern of 2,4-dinitrophenol (2,4-DNP) the major source for environmental release of 2,4-DNP is likely to be from production plants and chemical firms where the compound is used as an intermediate. It is possible that 2,4-DNP may also be produced via microbial or photodegradation of compounds which contain the dinitrophenol moiety, such as parathion (Gomaa and Faust, 1972). 2,4-DNP has also been identified as an impurity in technical preparations of the herbicide DNPP (2-isopropyl-4,6-dinitrophenol) by Mosinska and Kotarski (1972).

The physical and chemical properties of the dinitrophenol isomers are summarized in Table 2.

Trinitrophenols

Six isomeric forms of trinitrophenol are possible, distinguished by the position of the nitro- groups relative to the hydroxy group on the six carbon benzene ring. The five isomers are: 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-, and 3,4,5-trinitrophenol. Production volumes for the trinitrophenols are not available. Usage of the trinitrophenol isomers is apparently limited to 2,4,6-trinitrophenol, otherwise known as picric acid. In fact, a comprehensive search of the literature failed to detect a single citation dealing with any of the trinitrophenol isomers except picric acid. Consequently, the only information on these isomers presented in this document are the chemical and physical properties found in Table 3.

TABLE 2
Properties of Dinitrophenol Isomers*

Isomer	m.p. (°C)	K (at 25°C)	Water Solubility (g/l)	Density
2,3-Dinitrophenol	144	1.3×10^{-5}	2.2	1.681
2,4-Dinitrophenol	114-115 (sublimes)	1.0×10^{-4}	0.79	1.683
2,5-Dinitrophenol	104	7×10^{-6}	0.68	
2,6-Dinitrophenol	63.5	2.7×10^{-4}	0.42	
3,4-Dinitrophenol	134	4.3×10^{-5}	2.3	1.672
3,5-Dinitrophenol	122-123	2.1×10^{-4}	1.6	1.702

*Source: Harvey, 1959; Windholz, 1976; Weast, 1975.

TABLE 3
Properties of Trinitrophenols*

<u>2,3,4-Trinitrophenol</u>	
Molecular Weight	229.11
<u>2,3,5-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	119-120°C
<u>2,3,6-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	119°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Very Soluble
<u>2,4,5-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	96°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Soluble
<u>2,4,6-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	122-123°C
Boiling Point	Sublimates: Explodes at 300°C
Vapor Pressure	1 mm Hg at 195°C
Density	1.763 g/cm ³
Water Solubility	
Room Temperature	1.28 g/l
100°C	6.7 g/l

*Source: Windholz, 1976; Weast, 1975; Matsuguma, 1967.

According to Matsuguma (1967) picric acid has found usage as: a dye intermediate, explosive, analytical reagent, germicide, fungicide, staining agent and tissue fixative, tanning agent, photochemical, pharmaceutical, and a process material for the oxidation and etching of iron, steel and copper surfaces. The extent to which picric acid finds usage in any of these applications at the present time is unknown.

Dinitrocresols

Dinitro-ortho cresol is a yellow crystalline solid derived from o-cresol. There are six possible isomers but the 4,6-dinitro-o-cresol isomer is the only one of any commercial importance. In fact, a comprehensive search of the literature failed to reveal information on any of the other five dinitrocresol isomers.

4,6-Dinitro-o-cresol (hereafter referred to as DNOC) is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperature. Some important chemical and physical properties of DNOC are shown in Table 4.

The U.S. Environmental Protection Agency (U.S. EPA) has no record of DNOC being currently manufactured in the United States for use as an agricultural chemical. Imports of DNOC have also decreased in recent years; from 217,899 lbs. in 1972 to 146,621 lbs. in 1973 and then to 30,442 lbs. in 1976 (National Institute for Occupational Safety and Health (NIOSH), 1978). Since DNOC is not manufactured in the U.S., pesticide formulators and sprayers are the major groups with potential occupational exposure to DNOC.

DNOC is used primarily as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant

TABLE 4

Properties of 4,6-Dinitro-*o*-cresol*

Molecular Weight	198.13
Appearance	Yellow Solid
Melting Point	85.8°C
Vapor Pressure	0.000052 mm Hg at 20°C
Water Solubility	100 mg/l at 20°C
pKa	4.46

*Source: Windholz, 1976; Weast, 1975; Matsuguma, 1967.

season. NIOSH (1978) estimates that 3,000 workers in the U.S. are potentially exposed to DNOC. In view of the small amount of DNOC used in the U.S., exposure of the general public is expected to be minimal.

In general, few data are available regarding the breakdown of nitrophenols by natural communities of microorganisms. A number of researchers have isolated microorganisms capable of using nitrophenols as a sole source of carbon in pure culture (Simpson and Evans, 1953; Raymond and Alexander, 1971; Chambers, et al. 1963; Guillaume, et al. 1963). However, the significance of such studies as related to the stability of nitrophenols in the environment is not known.

Several investigators have shown that individual species of aerobic and anaerobic bacteria, including Azotobacter chroococcum and Clostridium butyrium, and the fungus Fusarium, are capable of reducing 2,4-dinitrophenol in culture (Radler, 1955; Lehmer, 1956; Madhosingh, 1961). However, the precise pathway for metabolic degradation is not known. Jensen and Lautrup-Larson (1967) found that Arthrobacter simplex, Pseudomonas, and Arthrobacter were able to metabolize 2,4-dinitrophenol and 2,4,6-trinitrophenol, forming nitrite.

The actual degradation pathway of dinitro-*o*-cresol has been investigated by Tewfik and Evans (1966) in pure cultures of microorganisms. It was reported that in Pseudomonas sp. degradation proceeded by way of formation of an aminocresol. In Arthrobacter simplex, a hydroxylated catechol is formed prior to ring cleavage.

The significance of such studies as related to the stability of nitrophenols in the environment is not known. Certain investigators have postulated that ambient nitrophenol concentrations may be too low to induce the appropriate microbial enzymes necessary to facilitate population growth and metabolism of the compounds (U.S. EPA, 1976).

Information regarding the mobility and persistence of nitrophenols in natural soil and water environments is limited. Based upon experimentally determined solubilities and sorption characteristics, the persistence of some of the nitrophenols might be estimated. For example, although 2-nitrophenol is soluble in water, it has also been shown to be strongly attracted through hydrogen bonding to montmorillonite clays, perhaps reducing its movement through the groundwater regime (Saltzman and Yariv, 1975; Yariv, et al. 1966). However, these estimates do not consider the data available in the literature on microbial, photolytic, and oxidative degradation.

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INTRODUCTION

Although fish and invertebrate acute toxicity data and plant toxicity data are available for the groups of organic compounds which contain various numbers of nitro groups substituted into the aromatic ring of a phenol or cresol, collectively referred to as nitrophenols, there are only limited data available for any individual nitrophenol. There are no data available dealing with chronic effects of any nitrophenol on freshwater aquatic organisms, and no suitable substitute chronic value can be determined from available toxicity information. The limited data available preclude deriving a criterion for any of the individual nitrophenol compounds. The derivation of a single criterion which would protect freshwater aquatic organisms from all nitrophenols is also impractical because of the wide difference in toxicity of individual nitrophenols.

The saltwater data base available for the various nitrophenols is also limited. For 4-nitrophenol there are acute test results for one fish and one invertebrate species, and one fish chronic test. In addition, there are two invertebrate lethal threshold values. The data base for 2,4-dinitrophenol consists of acute tests, one algal test, and miscellaneous effects on one invertebrate species. Information on 2,4,6-trinitrophenol is even more limited, consisting of acute results for one fish, one algal, and one invertebrate species.

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

EFFECTS

Acute Toxicity

The data base for freshwater invertebrate species (Table 1) contains seven data points for four nitrophenol compounds with two invertebrate species. An unspecified dinitromethylphenol (reported by Sanders and Cope, 1968 as dinitrocresol) is the most toxic compound with an LC_{50} value of 320 $\mu\text{g}/\text{l}$ for a stonefly, Pteronarcys californica. This compound is followed in order of decreasing toxicity by 2,4-dinitro-6-methylphenol, 2,4-dinitrophenol, 4-nitrophenol, and 2,4,6-trinitrophenol. The 2,4-dinitro-6-methylphenol LC_{50} for Daphnia magna is 3,120 $\mu\text{g}/\text{l}$ (U.S. EPA, 1978). It appears that stoneflies may be more sensitive to dinitromethylphenol than are daphnids, although this is impossible to verify since the compound tested with stoneflies is inadequately identified and may be a slightly different compound than that tested with daphnids. For 2,4-dinitrophenol, the two LC_{50} values for daphnids are quite close and are 4,710 $\mu\text{g}/\text{l}$ (Kopperman, et al. 1974) and 4,090 $\mu\text{g}/\text{l}$ (U.S. EPA, 1978). The toxicity of 4-nitrophenol to daphnids shows greater variation between investigators with reported LC_{50} values of 8,396 $\mu\text{g}/\text{l}$ (Kopperman, et al. 1974) and 21,900 $\mu\text{g}/\text{l}$ (U.S. EPA, 1978). The least toxic nitrophenol to daphnids is 2,4,6-trinitrophenol with a reported LC_{50} value of 84,700 $\mu\text{g}/\text{l}$ (U.S. EPA, 1978).

The freshwater fish acute toxicity data base (Table 1) consists of seven LC_{50} values for four nitrophenols and two fish species. Although differences in test methods make comparisons difficult, it appears bluegills are more sensitive than fathead minnows with all three nitrophenols for which data are available for both species. Comparisons of LC_{50} values for fishes indicate that 2,4-dinitro-6-methylphenol is the most toxic nitrophenol with LC_{50} values of 230 $\mu\text{g}/\text{l}$ (U.S. EPA, 1978) and 2,030 $\mu\text{g}/\text{l}$

(Phipps, et al. Manuscript) for the bluegill and fathead minnow, respectively; 2,4-dinitro-6-methylphenol is followed in order of decreasing toxicity by 2,4-dinitrophenol, 4-nitrophenol, and 2,4,6-trinitrophenol. The largest variation in species sensitivity occurred with 2,4-dinitrophenol where LC_{50} values are 620 $\mu\text{g/l}$ for bluegills (U.S. EPA, 1978) and 16,700 $\mu\text{g/l}$ for fathead minnows (Phipps, et al. Manuscript). The LC_{50} values reported for 4-nitrophenol for bluegills and fathead minnows are 8,280 $\mu\text{g/l}$ (U.S. EPA, 1978) and 60,500 $\mu\text{g/l}$ (Phipps, et al. Manuscript), respectively. The high 2,4,6-trinitrophenol LC_{50} of 167,000 $\mu\text{g/l}$ for bluegills (U.S. EPA, 1978) indicates the toxicity of nitrophenols does not increase directly with increasing nitro-group substitution.

The order of toxicity of the four nitrophenol compounds tested with both freshwater fish and invertebrate species is the same, and it appears from the limited data available that there are no large sensitivity differences among these species with any of these nitrophenols. Invertebrate toxicity values for 4-nitrophenol and 2,4-dinitrophenol fall between those calculated for bluegills and fathead minnows. Daphnia magna appeared slightly more sensitive to 2,4,6-trinitrophenol than were bluegills, and slightly less sensitive to 2,4-dinitro-6-methylphenol than were fathead minnows.

As seen in Table 1 for the saltwater acute data, 4-nitrophenol resulted in a 96-hour LC_{50} of 7,170 $\mu\text{g/l}$ with mysid shrimp, whereas the sheepshead minnow showed greater resistance with a 96-hour LC_{50} value of 27,100 $\mu\text{g/l}$. Both the mysid shrimp and the herring embryos exhibited greater sensitivity to 2,4-dinitrophenol (96-hour LC_{50} values of 4,850 and 5,500 $\mu\text{g/l}$, respectively) than did the sheepshead minnow at 29,400 $\mu\text{g/l}$. Of the three nitrophenols tested, 2,4,6-trinitrophenol appeared to be the least toxic, producing 96-hour LC_{50} values of 19,700 $\mu\text{g/l}$ for the mysid shrimp

and 134,000 µg/l for the sheepshead minnow. This nitrophenol was also the least toxic of the tested compounds to freshwater fish and invertebrate species. Although the invertebrate species was consistently more sensitive to all these compounds than was the sheepshead minnow, the herring embryos also were very sensitive to the toxic effects of 2,4-dinitrophenol.

Chronic Toxicity

There are no data available on the chronic effects of any of the various nitrophenol compounds on freshwater aquatic life.

The chronic effects of 4-nitrophenol and 2,4-dinitrophenol on hatching and survival in an early life stage test with the sheepshead minnow have been determined (U.S. EPA, 1978). Chronic values of 12,650 and 7,900 µg/l were obtained for 4-nitrophenol and 2,4-dinitrophenol, respectively (Table 2). Although 4-nitrophenol may be somewhat less toxic in the chronic test, the compounds were of similar acute toxicity to the sheepshead minnow (Table 1).

Acute-chronic ratios for these nitrophenols may be derived for the sheepshead minnow. For 4-nitrophenol the acute-chronic ratio is 2.1, and for 2,4-dinitrophenol the ratio is 3.7.

There are no nitrophenol chronic effects data for any saltwater invertebrate species.

Plant Effects

Freshwater plant toxicity values (Table 3) are lower, in certain instances, than acute LC₅₀ values for fish and invertebrate species. Exposure to 4-nitrophenol produced toxic effects in Chlorella vulgaris at 6,950 µg/l (Dedonder and Van Sumere, 1971) and in Selenastrum capricornutum at 4,190 µg/l (U.S. EPA, 1978). These plant effect levels for 4-nitrophenol are both below the lowest fish or invertebrate LC₅₀ values for this compound. A 50 percent reduction in chlorophyll a also occurred in Selenastrum

cabriornutum in 96 hours at a 2,4,6-trinitrophenol concentration (41,700 $\mu\text{g/l}$) that is below the lowest LC_{50} value for fish or invertebrate species. As observed with fish and invertebrate species, 2,4,6-trinitrophenol is less toxic than 4-nitrophenol and 2,4-dinitrophenol to Selenastrum cabriornutum (U.S. EPA, 1978).

Dedonder and Van Sumere (1971) determined that 9,200 $\mu\text{g/l}$ of 2,4-dinitrophenol caused a 70 percent growth inhibition in Chlorella vulgaris in 80 hours, although this concentration caused only a 25 percent growth inhibition in 160 hours.

Results of tests which examined the relative toxicity of the three isomeric forms of mononitrophenols to an alga (Huang and Gloyna, 1967) indicated that chlorophyll synthesis in Chlorella pyrenoidosa was inhibited to a point below initial control levels at concentrations of 25,000 $\mu\text{g/l}$ by 4-nitrophenol, 35,000 $\mu\text{g/l}$ by 2-nitrophenol, and 50,000 $\mu\text{g/l}$ by 3-nitrophenol. Studies with three species of algae indicate that 4-nitrophenol is slightly more toxic to plants than is 2,4-dinitrophenol (Table 3). The one exception to this toxicity trend was observed by Simon and Blackman (1953), who found that 50 percent growth reduction in duckweed, Lemna minor, occurred at 2,4-dinitrophenol and 4-nitrophenol concentrations of 1,472 $\mu\text{g/l}$ and 9,452 $\mu\text{g/l}$, respectively. However, these observed effects on duckweed occurred under conditions of low pH (5.2 and 5.4) and effects at a more neutral pH were not measured.

All of the available freshwater plant effects data for nitrophenols are based on unmeasured concentrations and therefore there is no Freshwater Final Plant Value.

Only one saltwater algal species was examined for its response to three nitrophenols (U.S. EPA, 1978). The effects of these compounds on Skeletone-ma costatum are summarized in Table 3. The 96-hour LC_{50} concentrations of

4-nitrophenol (about 7,000 $\mu\text{g/l}$) and 2,4-dinitrophenol (about 95,000 $\mu\text{g/l}$) were very similar for effects on both chlorophyll a production and reduction in cell numbers. However, 2,4,6-trinitrophenol seemed to have a greater effect on chlorophyll a, with a 96-hour EC_{50} of 62,7000 $\mu\text{g/l}$, than on cell numbers where the effective concentration was 141,000 $\mu\text{g/l}$. Skeletonema costatum was most sensitive to 4-nitrophenol.

Residues

No measured, steady-state bioconcentration factors are available for saltwater or freshwater organisms and any nitrophenol.

Miscellaneous

No miscellaneous data were found that would be a suitable substitute for a Final Chronic Value for any nitrophenol compound (Table 4), although some data are the lowest acute values or the only acute values available for several nitrophenol compounds. Bringmann and Kuhn (1978) calculated the 8-day toxicity thresholds (the pollutant concentration causing the onset of cell multiplication inhibition) for nine nitrophenol compounds with two algal species, Microcystis aeruginosa and Scenedesmus quadricauda. Data from the most sensitive species tested in this study are the lowest plant values available for 2-nitrophenol (4,300 $\mu\text{g/l}$), 3-nitrophenol (7,600 $\mu\text{g/l}$), 2,4,6-trinitrophenol (40,000 $\mu\text{g/l}$), and 2,4-dinitro-6-methylphenol (150 $\mu\text{g/l}$) and are the only plant data available for 2-nitro-p-cresol (3,800 $\mu\text{g/l}$), 4-nitro-m-cresol (7,000 $\mu\text{g/l}$), and 6-nitro-m-cresol (7,000 $\mu\text{g/l}$). In another study, Bringmann and Kuhn (1977) determined the 24-hour LC_{50} values for the same nine nitrophenols with the cladoceran, Daphnia magna. This study provides the only invertebrate or fish acute data available for 2-nitro-p-cresol (130,000 $\mu\text{g/l}$), 4-nitro-m-cresol (33,000 $\mu\text{g/l}$), and 6-nitro-m-cresol (43,000 $\mu\text{g/l}$), and also provides a toxicity comparison of the

three isomeric forms of mononitrophenols with daphnids. They found 4-nitrophenol to be the most toxic compound to daphnids, followed in order of decreasing toxicity by 3-nitrophenol and 2-nitrophenol. Gersdorff (1939) also found the same relative order of toxicity for the three mononitrophenols with goldfish (8,000 $\mu\text{g/l}$ for 4-nitrophenol, 24,000 $\mu\text{g/l}$ for 3-nitrophenol, and 33,300 $\mu\text{g/l}$ for 2-nitrophenol). While these are not LC_{50} values, they represent the lowest acute effects available for these three mononitrophenol compounds. Although the relative order of toxicity is the same for the three mononitrophenols with both fish and invertebrate species, the toxicity order appeared to be slightly different in the alga, Chlorella pyrenoidosa (Huang and Gloyna, 1967, Table 3).

Juvenile Atlantic salmon were much more sensitive to 2,4-dinitrophenol, with a lethal threshold value of 700 $\mu\text{g/l}$ (Zitko, et al. 1976). (Lethal threshold is the geometric mean of the highest concentration with no deaths and the next higher concentration at which all animals died). The authors tested a variety of compounds with both freshwater and saltwater species. They did not specify whether the Atlantic salmon was tested in salt or freshwater, but the size of the tested organism leads us to believe that the test was probably conducted in freshwater.

The only saltwater datum for 2-nitrophenol is listed in Table 4. This result is very similar to that for 4-nitrophenol and the shrimp, Crangon septemspinosus. The soft shell clam responded to the toxic effects of 4-nitrophenol at about the same concentration (McLeese, et al. 1979). 2,4-Dinitrophenol also adversely affected sperm and embryo of two sea urchin species at concentrations as low as 46,000 $\mu\text{g/l}$.

Summary

For the four nitrophenols for which freshwater acute data are available, the order of most to least toxic is 2,4-dinitro-6-methylphenol, 2,4-dinitrophenol, 4-nitrophenol, and 2,4,6-trinitrophenol. Acute LC_{50} values ranged from a low of 230 $\mu\text{g/l}$ for bluegills exposed to 2,4-dinitro-6-methylphenol to a high of 167,000 $\mu\text{g/l}$ for bluegills exposed to 2,4,6-trinitrophenol. There are no freshwater chronic data available for any nitrophenol. Plant values and miscellaneous data for the various nitrophenols showed that effects occurred at exposure concentrations ranging from a low of 150 $\mu\text{g/l}$ for 2,4-dinitro-6-methylphenol, based on an 8-day toxicity threshold with algae, to a high of 210,000 $\mu\text{g/l}$ for 2-nitrophenol, based on a 24-hour LC_{50} with daphnids.

For the three nitrophenols having a saltwater acute toxicity data base, the mysid shrimp was consistently more sensitive than the sheepshead minnow. In comparing the acute toxicity of these three compounds, the least toxic for both organisms was 2,4,6-trinitrophenol. Acute and early life stage tests conducted on the sheepshead minnow yielded acute-chronic ratios of 2.1 for 4-nitrophenol and 3.7 for 2,4-dinitrophenol. There are no chronic data for 2,4,6-trinitrophenol.

Plant values and miscellaneous data for the various nitrophenols showed a concentration range of effects from a low of 7,370 $\mu\text{g/l}$ for 4-nitrophenol on chlorophyll a production in an alga to a high of 141,000 $\mu\text{g/l}$ for an effect of 2,4,6-trinitrophenol on cell numbers of the same species.

CRITERIA

The available data for nitrophenols indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 230 $\mu\text{g/l}$ and would occur at lower concentrations among species that are more sensitive

than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive freshwater aquatic life but toxicity to one species of algae occurs at concentrations as low as 150 $\mu\text{g}/\text{l}$.

The available data for nitrophenols indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 4,350 $\mu\text{g}/\text{l}$ and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of nitrophenols to sensitive saltwater aquatic life.

Table 1. Acute values for nitrophenols

<u>Species</u>	<u>Method[#]</u>	<u>Chemical</u>	<u>LC50/EC50 (µg/l)</u>	<u>Species Mean Acute Value (µg/l)</u>	<u>Reference</u>
<u>FRESHWATER SPECIES</u>					
<u>Cladoceran, Daphnia magna</u>	S, U	4-nitrophenol	8,396	-	Kopperman, et al. 1974
<u>Cladoceran, Daphnia magna</u>	S, U	4-nitrophenol	21,900	13,560	U.S. EPA, 1978
<u>Cladoceran, Daphnia magna</u>	S, U	2,4-dinitro- phenol	4,710	-	Kopperman, et al. 1974
<u>Cladoceran, Daphnia magna</u>	S, U	2,4-dinitro- phenol	4,090	4,389	U.S. EPA, 1978
<u>Cladoceran, Daphnia magna</u>	S, U	2,4,6-trinitro- phenol	84,700	84,700	U.S. EPA, 1978
<u>Cladoceran, Daphnia magna</u>	S, U	2,4-dinitro-6- methylphenol	3,120	3,120	U.S. EPA, 1978
<u>Stonefly (naiad), Pteronarcys californica</u>	S, U	Dinitromethyl- phenol**	320	320	Sanders & Cope, 1968
<u>Fathead minnow (juvenile), Pimephales promelas</u>	FT, M	4-nitrophenol	60,500	60,500	Philpps, et al. Manuscript
<u>Fathead minnow (juvenile), Pimephales promelas</u>	FT, M	2,4-dinitro- phenol	16,700	16,700	Philpps, et al. Manuscript
<u>Fathead minnow (juvenile), Pimephales promelas</u>	FT, M	2,4-dinitro-6- methylphenol**	2,030	2,030	Philpps, et al. Manuscript
<u>Bluegill, Lepomis macrochirus</u>	S, U	4-nitrophenol	8,280	8,280	U.S. EPA, 1978
<u>Bluegill, Lepomis macrochirus</u>	S, U	2,4-dinitro- phenol	620	620	U.S. EPA, 1978
<u>Bluegill, Lepomis macrochirus</u>	S, U	2,4,6-trinitro- phenol	167,000	167,000	U.S. EPA, 1978

Table 1. (Continued)

<u>Species</u>	<u>Method*</u>	<u>Chemical</u>	<u>LC50/LC50 (µg/l)</u>	<u>Species Mean Acute Value (µg/l)</u>	<u>Reference</u>
<u>Bluegill, Lepomis macrochirus</u>	S, U	2,4-dinitro-6- methylphenol	230	230	U.S. EPA, 1978
<u>SALT WATER SPECIES</u>					
<u>Mysid shrimp, Mysidopsis bahia</u>	S, U	4-nitrophenol	7,170	7,170	U.S. EPA, 1978
<u>Mysid shrimp, Mysidopsis bahia</u>	S, U	2,4-dinitro- phenol	4,850	4,850	U.S. EPA, 1978
<u>Mysid shrimp, Mysidopsis bahia</u>	S, U	2,4,6-trinitro- phenol	19,700	19,700	U.S. EPA, 1978
<u>Herring (embryo), Clupea harengus</u>	S, U	2,4-dinitro- phenol	5,500	5,500	Rosenthal & Stelzer, 1970
<u>Sheepshead minnow, Cyprinodon variegatus</u>	S, U	4-nitrophenol	27,100	27,100	U.S. EPA, 1978
<u>Sheepshead minnow, Cyprinodon variegatus</u>	S, U	2,4-dinitro- phenol	29,400	29,400	U.S. EPA, 1978
<u>Sheepshead minnow, Cyprinodon variegatus</u>	S, U	2,4,6-trinitro- phenol	134,000	134,000	U.S. EPA, 1978

* S = static, FT = flow-through, U = unmeasured, M = measured

**Authors reported results as 4,6-dinitro-o-cresol or as only dinitrocresol (Sanders and Cope, 1968).

Table 2. Chronic values for nitrophenols (U.S. EPA, 1978)

<u>Species</u>	<u>Method</u> ^a	<u>Chemical</u>	<u>Limits</u> ($\mu\text{g/l}$)	<u>Chronic Value</u> ($\mu\text{g/l}$)
<u>SALTWATER SPECIES</u>				
<u>Sheepshead minnow,</u> <u>Cyprinodon variegatus</u>	ELS	4-nitrophenol	10,000- 16,000	12,650
<u>Sheepshead minnow,</u> <u>Cyprinodon variegatus</u>	ELS	2,4-dinitro- phenol	5,200- 12,000	7,900

* ELS = early life stage

<u>Acute-Chronic Ratio</u>				
<u>Species</u>	<u>Chemical</u>	<u>Acute Value</u> ($\mu\text{g/l}$)	<u>Chronic Value</u> ($\mu\text{g/l}$)	<u>Ratio</u>
<u>Sheepshead minnow,</u> <u>Cyprinodon variegatus</u>	4-nitrophenol	27,100	12,650	2.1
<u>Sheepshead minnow,</u> <u>Cyprinodon variegatus</u>	2,4-dinitro- phenol	29,400	7,900	3.7

Table 3. Plant values for nitrophenols

<u>Species</u>	<u>Chemical</u>	<u>Effect</u>	<u>Result</u> ($\mu\text{g/l}$)	<u>Reference</u>
<u>FRESHWATER SPECIES</u>				
Alga, <u>Chlorella pyrenoidosa</u>	2-nitrophenol	Inhibition of chlorophyll synthesis after 3 days	35,000	Huang & Gloyna, 1967
Alga, <u>Chlorella pyrenoidosa</u>	3-nitrophenol	Inhibition of chlorophyll synthesis after 3 days	50,000	Huang & Gloyna, 1967
Alga, <u>Chlorella pyrenoidosa</u>	4-nitrophenol	Inhibition of chlorophyll synthesis after 3 days	25,000	Huang & Gloyna, 1967
Alga, <u>Chlorella pyrenoidosa</u>	2,4-dinitrophenol	Inhibition of chlorophyll synthesis after 3 days	50,000	Huang & Gloyna, 1967
Alga, <u>Chlorella pyrenoidosa</u>	2,4-dinitro-6-methylphenol*	Inhibition of chlorophyll synthesis after 3 days	50,000	Huang & Gloyna, 1967
Alga, <u>Chlorella vulgaris</u>	4-nitrophenol	50% growth inhibition in 80 hrs	6,950	Dedonder & Van Sumere, 1971
Alga, <u>Chlorella vulgaris</u>	2,4-dinitrophenol	70% growth inhibition in 80 hrs	9,200	Dedonder & Van Sumere, 1971
Alga, <u>Chlorella vulgaris</u>	2,4-dinitrophenol	25% growth inhibition in 160 hrs	9,200	Dedonder & Van Sumere, 1971
Alga, <u>Selenastrum capricornutum</u>	4-nitrophenol	96-hr EC50, chlorophyll <u>a</u>	4,190	U.S. EPA, 1978
Alga, <u>Selenastrum capricornutum</u>	2,4-dinitrophenol	96-hr EC50, chlorophyll <u>a</u>	9,200	U.S. EPA, 1978

Table 3. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
Alga, <u>Solenastrum capricornutum</u>	2,4,6-trinitro- phenol	96-hour EC50 chlorophyll <u>a</u>	41,700	U.S. EPA, 1978
Duckweed, <u>Lemna minor</u>	2-nitrophenol	50% growth reduction	62,550	Simon & Blackman, 1953
Duckweed, <u>Lemna minor</u>	4-nitrophenol	50% growth reduction	9,452	Simon & Blackman, 1953
Duckweed, <u>Lemna minor</u>	2,4-dinitro- phenol	50% growth reduction	1,472	Simon & Blackman, 1953
<u>SALTWATER SPECIES</u>				
Alga, <u>Skeletonema costatum</u>	4-nitrophenol	96-hr EC50, chlorophyll <u>a</u>	7,370	U.S. EPA, 1978
Alga, <u>Skeletonema costatum</u>	4-nitrophenol	96-hr EC50, cell number	7,570	U.S. EPA, 1978
Alga, <u>Skeletonema costatum</u>	2,4-dinitro- phenol	96-hr EC50, chlorophyll <u>a</u>	93,200	U.S. EPA, 1978
Alga, <u>Skeletonema costatum</u>	2,4-dinitro- phenol	96-hr EC50, cell number	98,700	U.S. EPA, 1978
Alga, <u>Skeletonema costatum</u>	2,4,6-trinitro- phenol	96-hr EC50, chlorophyll <u>a</u>	62,700	U.S. EPA, 1978
Alga, <u>Skeletonema costatum</u>	2,4,6-trinitro- phenol	96-hr EC50, cell number	141,000	U.S. EPA, 1978

* Authors reported results as 4,6-dinitro-o-cresol.

Table 4. Other data for nitrophenols

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result</u> ($\mu\text{g/l}$)	<u>Reference</u>
FRESHWATER SPECIES					
<u>Alga,</u> <u>Chlamydomonas sp.</u>	Dinitrophenol	30 sec	50% inhibition of flagellar motility	18,400	Marcus & Mayer, 1963
<u>Alga,</u> <u>Microcystis aeruginosa</u>	2-nitrophenol	8 days	Toxicity threshold*	27,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	2-nitrophenol	8 days	Toxicity threshold*	4,300	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Microcystis aeruginosa</u>	3-nitrophenol	8 days	Toxicity threshold*	17,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	3-nitrophenol	8 days	Toxicity threshold*	7,600	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Microcystis aeruginosa</u>	4-nitrophenol	8 days	Toxicity threshold*	56,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	4-nitrophenol	8 days	Toxicity threshold*	7,400	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Microcystis aeruginosa</u>	2,4-dinitrophenol	8 days	Toxicity threshold*	33,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	2,4-dinitrophenol	8 days	Toxicity threshold*	16,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Microcystis aeruginosa</u>	2,4,6-trinitrophenol	8 days	Toxicity threshold*	40,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	2,4,6-trinitrophenol	8 days	Toxicity threshold*	61,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Microcystis aeruginosa</u>	2-nitro-p-cresol	8 days	Toxicity threshold*	32,000	Bringmann & Kuhn, 1978
<u>Alga,</u> <u>Scenedesmus quadricauda</u>	2-nitro-p-cresol	8 days	Toxicity threshold*	3,800	Bringmann & Kuhn, 1978

Table 4. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
Alga, <u>Microcystis aeruginosa</u>	4-nitro-m-cresol	8 days	Toxicity threshold*	13,000	Bringmann & Kuhn, 1978
Alga, <u>Scenedesmus quadricauda</u>	4-nitro-m-cresol	8 days	Toxicity threshold*	7,000	Bringmann & Kuhn, 1978
Alga, <u>Microcystis aeruginosa</u>	6-nitro-m-cresol	8 days	Toxicity threshold*	34,000	Bringmann & Kuhn, 1978
Alga, <u>Scenedesmus quadricauda</u>	6-nitro-m-cresol	8 days	Toxicity threshold*	7,000	Bringmann & Kuhn, 1978
Alga, <u>Microcystis aeruginosa</u>	2,4-dinitro-6-methylphenol**	8 days	Toxicity threshold*	150	Bringmann & Kuhn, 1978
Alga, <u>Scenedesmus quadricauda</u>	2,4-dinitro-6-methylphenol**	8 days	Toxicity threshold*	13,000	Bringmann & Kuhn, 1978
Amoeba, <u>Amoeba proteus</u>	Dinitrophenol	24 hrs	46% reduction in amoeba containing golgi bodies	92,000	Flickinger, 1972
Amoeba, <u>Amoeba proteus</u>	Dinitrophenol	48 hrs	18% mortality	92,000	Flickinger, 1972
Cladoceran, <u>Daphnia magna</u>	2-nitrophenol	24 hrs	LC50	210,000	Bringmann & Kuhn, 1977
Cladoceran, <u>Daphnia magna</u>	3-nitrophenol	24 hrs	LC50	39,000	Bringmann & Kuhn, 1977
Cladoceran, <u>Daphnia magna</u>	4-nitrophenol	24 hrs	LC50	35,000	Bringmann & Kuhn, 1977
Cladoceran, <u>Daphnia magna</u>	2,4-dinitrophenol	24 hrs	LC50	19,000	Bringmann & Kuhn, 1977
Cladoceran, <u>Daphnia magna</u>	2,4,6-trinitrophenol	24 hrs	LC50	145,000	Bringmann & Kuhn, 1977
Cladoceran, <u>Daphnia magna</u>	2-nitro-p-cresol	24 hrs	LC50	130,000	Bringmann & Kuhn, 1977

Table 4. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
<u>Cladoceran, Daphnia magna</u>	4-nitro-m- cresol	24 hrs	LC50	33,000	Bringmann & Kuhn, 1977
<u>Cladoceran, Daphnia magna</u>	6-nitro-m- cresol	24 hrs	LC50	43,000	Bringmann & Kuhn, 1977
<u>Cladoceran, Daphnia magna</u>	2,4-dinitro-6- methylphenol**	24 hrs	LC50	6,600	Bringmann & Kuhn, 1977
<u>Southern bullfrog (tadpole), Rana grylio</u>	2,4-dinitro- phenol	7 hrs	Increased respiration	5,520	Lewis & Frieden, 1959
<u>Atlantic salmon (juvenile), Salmo salar</u>	2,4-dinitro- phenol	96 hrs	Lethal threshold value	700	Zitko, et al. 1976
<u>Goldfish, Carassius auratus</u>	2-nitrophenol	8 hrs	38% mortality	33,300	Gersdorff, 1939
<u>Goldfish, Carassius auratus</u>	3-nitrophenol	8 hrs	53% mortality	24,000	Gersdorff, 1939
<u>Goldfish, Carassius auratus</u>	4-nitrophenol	8 hrs	42% mortality	8,000	Gersdorff, 1939
<u>Bluegill (juvenile), Lepomis macrochirus</u>	2-nitrophenol	24 hrs	LC50	66,900	Lanmering & Burbank, 1960
<u>Bluegill (juvenile), Lepomis macrochirus</u>	2-nitrophenol	48 hrs	LC50	46,300- 51,600	Lanmering & Burbank, 1960
<u>SALTWATER SPECIES</u>					
<u>Shrimp, Crangon septempinnosa</u>	2-nitrophenol	96 hrs	Lethal threshold value	32,900	McLeese, et al. 1979
<u>Shrimp, Crangon septempinnosa</u>	4-nitrophenol	96 hrs	Lethal threshold value	26,400	McLeese, et al. 1979
<u>Clam (soft-shell), Mya arenaria</u>	4-nitrophenol	96 hrs	Lethal threshold value	29,400	McLeese, et al. 1979

Table 4. (Continued)

<u>Species</u>	<u>Chemical</u>	<u>Duration</u>	<u>Effect</u>	<u>Result (µg/l)</u>	<u>Reference</u>
<u>Sea urchin (sperm), Strongylocentrotus purpuratus</u>	2,4-dinitrophenol	1+ hrs	Inhibit respiration, motility	92,000	Bernstein, 1955
<u>Sea urchin (embryo), Pseudocentrotus depressus</u>	2,4-dinitrophenol	2 hrs	Abnormal cleavage	46,000	Kojima, 1960

* Toxicity threshold = the pollutant concentration causing the onset of cell multiplication inhibition.

**Authors reported results as 4,6-dinitro-p-cresol.

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MONONITROPHENOLS

Mammalian Toxicology and Human Health Effects

INTRODUCTION

Mononitrophenol has three isomeric forms, distinguished by the position of the nitro- group on the phenolic ring. Three isomeric forms are possible, namely 2-nitrophenol, 3-nitrophenol, and 4-nitrophenol. The compounds are also commonly referred to as o-nitrophenol, m-nitrophenol, and p-nitrophenol, respectively.

Commercial synthesis of 2-nitrophenol and 4-nitrophenol is accomplished through the hydrolysis of the appropriate chloronitrobenzene isomers with aqueous sodium hydroxide at elevated temperatures (Howard, et al. 1976). Production of 3-nitrophenol is achieved through the diazotization and hydrolysis of m-nitroaniline (Matsuguma, 1967). The mononitrophenol isomers are used in the United States primarily as intermediates for the production of dyes, pigments, pharmaceuticals, rubber chemicals, lumber preservatives, photographic chemicals, and pesticidal and fungicidal agents (U.S. Int. Trade Comm. 1976). As a result of this use pattern, the major source for environmental release of mononitrophenols is likely to be from production plants and chemical firms where the compounds are used as intermediates. The mononitrophenols may also be inadvertently produced via microbial or photodegradation of pesticides which contain mononitrophenol moieties. Approximately 10 to 15 million pounds of 2-nitrophenol are produced annually (Howard, et al. 1976) for uses including synthesis of o-aminophenol, o-nitroanisole, and other dye stuffs (Matsuguma, 1967; Howard, et al. 1976). Although production figures for 3-nitrophenol are not available, Hoecker, et al. (1977) estimate that production is less than one million pounds annually. 3-Nitrophenol is used in the manufacture of dye intermediates such as

anisidine and m-aminophenol (Kouris and Northcott, 1963; Matsuduma, 1967). 4-Nitrophenol is probably the most important of the mononitrophenols in terms of quantities used and potential environmental contamination. Demand for 4-nitrophenol was 35,000,000 pounds in 1976 and production is projected to increase to 41,000,000 pounds by 1980 (Chem. Market. Reporter, 1976). Most of the 4-nitrophenol produced (87 percent) is used in the manufacture of ethyl and methyl parathions. Other uses (13 percent) include the manufacture of dye-stuffs and n-acetyl-p-aminophenol (APAP) and leather treatments. A possible source of human exposure to 4-nitrophenol is as a result of microbial or photodegradation of the parathions. In vivo production of 4-nitrophenol following absorption of parathion or other pesticides by humans is another possible source of human exposure.

Physical and chemical properties of the mononitrophenols are summarized in Table 1 and Figure 1.

EXPOSURE

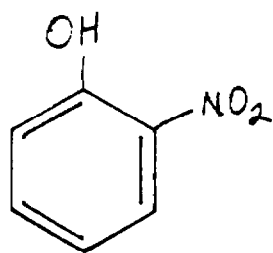
Ingestion from Water

Monitoring data on the presence of mononitrophenols in water are scant in the literature. Potential point sources for mononitrophenol contamination of water include industrial concerns engaged either in the manufacture of these compounds or their use as intermediates in chemical synthesis. Trifunovic, et al. (1971) detected unspecified levels of 4-nitrophenol in waste effluents from a parathion manufacturing plant. Webb, et al. (1973) reported a 4-nitrophenol level of 1.4 mg/l in the lagoon waste water from a chemical plant. Burnham, et al. (1972) detected 4-nitrophenol at levels of 0.2 mg/l in the potable water supply of Ames, Iowa. The source of the contamination was believed to be residues from a coal gas plant which ceased

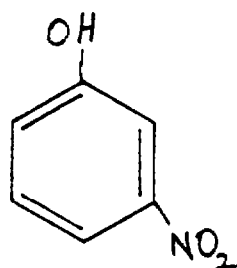
TABLE 1
Properties of Mononitrophenols*

	2-Nitrophenol	3-Nitrophenol	4-Nitrophenol
Formula	C ₆ H ₅ NO ₃	C ₆ H ₅ NO ₃	C ₆ H ₅ NO ₃
Molecular Weight	139.11	139.11	139.11
Melting Point (°C)	44-45	97	113-114
Boiling Point	214-216	279	279
Density	1.485	1.485	1.479
Water Solubility (g/l)	0.32 at 38°C 1.08 at 100°C	1.35 at 25°C 13.3 at 90°C	0.804 at 15°C 1.6 at 25°C
Vapor Pressure	1 mm Hg at 49.3°C		
K _a	7.5x10 ⁻⁸	5.3x10 ⁻⁹	7x10 ⁻⁸

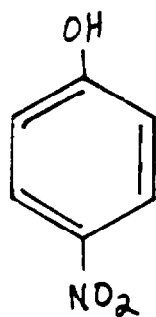
*Sources: Windholz, 1976; Weast, 1975; Matsuguma, 1967.



2-nitrophenol



3-nitrophenol



4-nitrophenol

FIGURE 1
Mononitrophenols

operation around 1930. 2-Nitrophenol was detected at unidentified levels in 2 river water samples and in 4 samples of chemical plant effluent; 3-nitrophenol was found in one chemical plant effluent sample (U.S. EPA, 1976). Systematic monitoring for mononitrophenols in the environment has not been done. It is reasonable to assume that measureable (although perhaps transient) levels of the mononitrophenols may be present in localized areas where organophosphate pesticides are in use.

Little data are available regarding the breakdown of mononitrophenols by natural communities of microorganisms. Alexander and Lustigman (1966) studied the degradation of mononitrophenols by a mixed population of soil microorganisms. The inoculum was derived from a suspension of Niagara silt loam soil. Their results indicated that 2-nitrophenol was more resistant to degradation than either 3-nitrophenol or 4-nitrophenol. Utilizing the absorbancy of small soil inoculums to estimate the loss of mononitrophenol, 3-nitrophenol was found to degrade completely within a 4-day period. 4-Nitrophenol degraded fully within a 16-day period, while 2-nitrophenol resisted degradation over a 64-day period.

Brebion, et al. (1967) examined the ability of microorganisms to attack 4-nitrophenol. The bacteria were derived from soil, water or mud and grown on a porous mineral bed and were cultivated in a mineral nutrient solution to which nitrophenols were added as the sole source of carbon. The experimental findings revealed no significant removal of the compound under these conditions.

In contrast to these reports, a number of investigators have found that the mononitrophenols are readily and rapidly degraded by acclimated populations of microorganisms. Tabak, et al. (1964) studied the ability of acclimated cultures derived from garden soil, compost, and river mud to de-

grade the mononitrophenols. Phenol-adapted bacteria derived from these sources were found to readily degrade all three monitrophenol isomers. Ninety-five percent degradation (measured spectrophotometrically) occurred within three to six days. Pitter (1976) reported greater than 95 percent degradation of the three mononitrophenol isomers in an acciimated sludge system. The nitrophenols served as the sole source of organic carbon and degradation was complete within 120 hours.

A recent study (Haller, 1978) reports on the ability of unacclimated microorganisms to degrade the mononitrophenols. Either sludge obtained from the primary settling tank of the city of Ithaca, N.Y. wastewater treatment plant or a Windsor loamy fine sand soil were used as the source of the inoculum. 2-Nitro-, 3-nitro-, and 4-nitrophenol (16 mg/l) were completely degraded in three to five days by the sludge system. Soil inocula degraded 16 mg of 3-nitrophenol/l in three to five days while a similar concentration of 2-nitrophenol and 4-nitrophenol required 7 to 14 days for complete degradation.

Although definitive conclusions cannot be derived from this limited number of studies, it appears that the mononitrophenols are readily and rapidly degraded by microbial populations present in the environment.

Ingestion from Food

No data were found demonstrating the presence of mononitrophenols in food. One possible source of mononitrophenol exposure for humans is through the food chain as a result of the ingestion of food crops contaminated with pesticides containing the nitrophenol moiety. The production of 4-nitrophenol by microbial metabolism of parathion is well documented (Munnecke and Hsieh, 1974, 1976; Siddaramappa, et al. 1973; Sethunathan and Yoshida, 1973; Katan and Lichtenstein, 1977; Sethunathan, 1973). Microbial metabolism of

fluoridifen (o-nitrophenyl, α,α,α -trifluoro-2-nitro-p-tolyl ether) results in the intermediate formation of 4-nitrophenol (Tewfik and Hamdi, 1975). The major degradation product of fluoridifen following uptake by peanut seedling roots was 4-nitrophenol (Eastin, 1971). 4-Nitrophenol was also detected in soybean roots following absorption of fluoridifen (Rogers, 1971). Photodecomposition of the herbicide nitrofen (2,4-dichlorophenyl-o-nitrophenyl ether) in aqueous suspensions under sunlight or simulated sunlight is characterized by rapid cleavage of the ether linkage to form 2,4-dichlorophenol and 4-nitrophenol (Nakagawa and Crosby, 1974). El-Refai and Hopkins (1966) have investigated the metabolic fate of parathion following foliar application or root absorption by bean plants, Phaseolus vulgaris. Detectable amounts of 4-nitrophenol were found in chloroform rinses of parathion-treated leaves after four days.

In another experiment, analysis of nutrient solutions containing parathion in which plants were grown for root absorption studies revealed 4-nitrophenol, paraoxon, and traces of degradation products. Since these compounds were also detected in control solution which did not contain plants, the authors concluded that possible photochemical oxidation processes had occurred in the aqueous medium. The authors believed that the 4-nitrophenol detected following foliar application of parathion was due to photochemical degradation. 4-Nitrophenol was not detected in bean plants following injection of parathion directly into the stems of bean plants (El-Refai and Hopkins, 1966).

4-Nitrophenol has also been detected as a photoalteration product of parathion following application to cotton plants (Joiner and Baetcke, 1973).

Archer (1974) has examined the dissipation of parathion and its metabolites from field spinach. Field plots were sprayed with either 0.5 or one

bound of active parathion per acre. Application recommendations for parathion are: not less than 14 days before harvest at the rate of 0.5 pounds active ingredient per acre. Spinach samples were analyzed daily for parathion residues and a number of known metabolites including 4-nitrophenol. Levels of 4-nitrophenol in the treated spinach (calculated on a fresh weight basis) are presented in Table 2. Unsprayed spinach control samples taken prior to any spray treatments contained 95 ug/kg 4-nitrophenol. The source of these residues was not determined. The effects of washing or blanching following harvest on the levels of 4-nitrophenol in human food crops are unknown.

4-Nitrophenol has been detected in human urine. Through analysis of human urine for residues of selected pesticides and their specific metabolites (Kutz, et al. 1978), the National Monitoring Program for Pesticides is collaborating with the U.S. Public Health Service in a three-year study to assess the exposure of the general population of the U.S. Based on the analysis of 416 samples collected from the general population, 4-nitrophenol was detected in 1.0 percent of the population. A mean urine level of 10.0 ug/l with a maximum value of 113.0 ug/l was reported. It is important to note that 4-nitrophenol residues in the urine need not (and probably do not) reflect exposure to 4-nitrophenol itself. Mononitrophenols are readily formed in vivo following exposure to a number of widely used pesticides.

Kutz, et al. (1978) considered exposure to methyl and ethyl parathion as the origin of the urinary 4-nitrophenol detected in their survey. However, if it is assumed that the reported urinary residues of 4-nitrophenol reflect direct exposure to the 4-nitrophenol, a pharmacokinetic estimate of exposure can be made. Assuming that the exposure to nitrophenol is steady-state, that 100 percent of the absorbed nitrophenol is excreted in the urine, and

TABLE 2

Levels of 4-Nitrophenol Following Application of Parathion to
Field Spinach at Two Different Application Rates*

Sample Day	4-Nitrophenol Residue ($\mu\text{g}/\text{kg}$) ^{a, b}	
	0.5 lb. Parathion/Acre	1.0 lb. Parathion/Acre
1	172	453
2	88	305
3	73	240
4	76	188
5	73	136
6	72	216
7	35	117
8	40	18
9	34	19
10	34	18
11	31	18
12	38	22
13	28	16
14	33	19

*Source: Archer, 1974.

^aCalculated on a fresh weight basis. Percent moisture from 86.4 to 89.2.

^bUnsprayed spinach control samples taken prior to any spray treatments contained 95 $\mu\text{g}/\text{kg}$.

that the average urine void is 1.4 l/day per 70 kg person, initial exposure levels can be estimated from residual levels found in urine. For example, the exposure level leading to the 1.0 µg/l residue can be calculated as follows:

$$\text{Exposure} = \frac{(10.0 \text{ } \mu\text{g nitrophenol/l}) (1.41 \text{ of urine/day})}{70 \text{ kg man}} = 0.02 \text{ } \mu\text{g/kg/day}$$

A similar calculation using the maximum urine residue level observed by Kutz, et al. (1978) (113 µg/l) gives an exposure of 2.26 µg/kg/day.

Knowles, et al. (1975) have demonstrated the production of a wide number of mononitrophenols including 2-nitrophenol in a model system simulating gastric digestion of smoked bacon. These studies, utilizing nitrosated liquid smoke, were conducted under conditions favorable to nitrosation, and since the temperature, pH, and duration employed approximated those encountered during gastric digestion, their results indicated that nitrosation of phenols in smoked bacon may occur in the stomach with resultant production of 2-nitrophenol.

Mononitrophenols may also be formed in vivo via metabolic degradation of pesticides such as parathion by humans. Excretion of 4-nitrophenol, a metabolite of the organophosphorous pesticides, parathion, methylparathion, O-ethyl O-(p-nitrophenyl) phenylphosphonothioic acid (EPN), and dicapthon is a good indicator of human exposure to these pesticides (Wolfe, et al. 1970; Broadway and Shafik, 1973; Elliott, et al. 1960; Roan, et al. 1969). 4-Nitrophenol has also been detected as a urinary metabolite of nitrobenzene in humans (Myslak, et al. 1971).

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various

aquatic animals seem to be proportional to the percent lipid in the tissue. Thus the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenol, but the equation " $\text{Log BCF} = (0.85 \text{ Log } P) - 0.70$ " can be used (Veith, et al. 1979) to estimate the steady-state BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). The log P values were obtained from Hansch and Leo (1979) or were calculated by the method described therein. The adjustment factor of $3.0/7.6 = 0.395$ is used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish in order to obtain the weighted average bioconcentration factor for the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans.

<u>Chemical</u>	<u>log P</u>	<u>Estimated Steady-State BCF</u>	<u>Weighted Average BCF</u>
2-nitrophenol	1.73	5.89	2.33
4-nitrophenol	1.91	8.38	3.31

Inhalation

Quantitative data were not found regarding the presence of mononitrophenols in air. Lao, et al. (1973) discussed the application of a gas chromatograph quadrupole mass spectrometer-data processor combination for routine analysis of air pollutants. During a sample run of urban ambient particulate matter (location not designated) these investigators identified the presence of 4-nitrophenol as well as a large number of other air pollutants. Quantitative data were not provided, however. Ambient air levels of 4-nitrophenol in a Boeing plant where the compound was used for the preservation of the cork surfaces of the Minuteman Missile were equal to or less than 0.05 mg/m^3 of air (Butler and Bodner, 1973).

4-Nitrophenol may be produced in the atmosphere through the photochemical reaction between benzene and nitrogen monoxide. Nojima, et al. (1975) irradiated a combination of benzene vapor and nitrogen monoxide gas for five hours with a xenon lamp and characterized the resulting photochemical products. The production of nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2,6-dinitrophenol was described by the authors. Identity of the compounds was confirmed using thin layer chromatography, gas chromatography, gas chromatography-mass spectrometry, and infrared spectrometry. The authors suggested that these nitro-compounds may be the cause of the characteristic symptoms of seriously stricken victims of photochemical smog in Japan including: headache, breathing difficulties, vomiting, rise in body temperature, and numbness in the extremities.

In a second paper (Nojima, et al. 1976), the photochemical reaction of toluene with nitrogen monoxide was investigated. It was felt that the products of photochemical reaction of toluene with nitrogen monoxide might be

more important in the production of photochemical smog since the concentration of toluene in urban air is higher than that of benzene. Compounds produced as a result of this reaction included o-cresol, m-nitrotoluene, 4-nitrophenol, 2-methyl-6-nitrophenol, 3-methyl-4-nitrophenol, 2-methyl-4-nitrophenol, and 2-methyl-4-nitrophenol. These compounds were identified by gas chromatography-mass spectrometry. In another experiment, the investigators examined the organic compounds present in rain. An analysis of rainwater yielded 4-nitrophenol, 2-methyl-6-nitrophenol and 2-methyl-4-nitrophenol. The authors suggested that the nitrophenols produced by the photochemical reactions, described above, dissolve in rain. It seems likely that in areas where severe photochemical smog exists, humans may be exposed to substantial levels of mononitrophenols. However, it is impossible to estimate the levels at which humans are exposed to these compounds via inhalation, based on available data.

Dermal

Roberts, et al. (1977) used human autopsy epidermal membranes in an in vitro system to determine the permeability of human skin to various compounds. Both 3-nitrophenol and 4-nitrophenol were shown to permeate the skin and to produce damage at threshold concentrations of 0.8 and 0.9 percent (w/v), respectively. According to Patty (1963); 2-nitrophenol may be absorbed through the intact skin. No information on possible human dermal exposure to the mononitrophenols was found.

PHARMACOKINETICS

Absorption and Distribution

Data specific to the absorption and tissue distribution of the mononitrophenols were not available. It is reasonable to assume, based on the rapid urinary elimination of the mononitrophenols, that the compounds may be restricted primarily to the blood and urine following absorption by humans.

Metabolism

Metabolism of the mononitrophenols probably occurs via one of three mechanisms in humans. The major route of mononitrophenol metabolism is undoubtedly conjugation and the resultant formation of either glucuronide or sulfate conjugates. Other possible routes of metabolism include reduction of amino-compounds or oxidation to dihydric-nitrophenols.

Sulfate and glucuronide conjugative processes are two of the major detoxification mechanisms in many species, including mammals (Quebbemann and Anders, 1973). In recent years, 4-nitrophenol has been used as a preferred substrate for biochemical analysis of the glucuronidation reaction in a wide number of species (Aitio, 1973; Sanchez and Tephly, 1974; Ranklin, 1974; Heenan and Smith, 1974; Yang and Wilkinson, 1971). This use reflects the simple techniques available for quantitating the disappearance of 4-nitrophenol and the synthesis of the glucuronide conjugate. The relevance of many of these in vitro studies towards an assessment of the metabolic fate of the mononitrophenols in humans is questionable; thus only those in vivo studies with direct relevance to the metabolic fate of mononitrophenols in humans or experimental animals are discussed here.

It has been known for some time that levels of the mixed function oxidases and the enzymes responsible for conjugation of many compounds are generally highest in the mammalian liver. Litterst, et al. (1975) assayed liver, lung, and kidney tissue from the rat, mouse, rabbit, hamster, and guinea pig for standard microsomal and soluble fraction enzymes involved in drug biotransformation. These studies included an analysis of glucuronide conjugation of 4-nitrophenol by these tissues. For all species, liver was the most active organ. Kidney and lung activities were usually 15 to 40

percent of that found in liver, with kidney slightly more active than lung. UDP-glucuronyl-transferase activity toward the acceptor, 4-nitrophenol, was higher in hamsters and rabbits than in other species.

Conjugation activity need not be constant even within the same species. Pulkkinen (1966b) noted that sulfate conjugation of 4-nitrophenol is decreased during pregnancy in rabbits. The author suggested that large amounts of estrogens may cause more protein binding, thus inhibiting the reaction. In another study (Pulkkinen, 1966a) it was noted that conjugation capacity increases with age in the rat, guinea pig, and man. The human fetus does not have a very high capacity to form sulfate or glucuronide conjugates of mononitrophenols or other compounds. In addition, Moldeus, et al. (1976) noted that the relative rate of glucuronide versus sulfate conjugation of 4-nitrophenol may depend on the levels of substrate present. In in vitro tests utilizing isolated rat liver cells, the investigators noted that at 4-nitrophenol concentrations of 25 μ M, the rate of glucuronide conjugation was low and over 75 percent of the conjugation products were found to be sulfates. The glucuronidation increased more rapidly than did the sulfate conjugation with increasing substrate conjugation. At 250 μ M, conjugation of 4-nitrophenol with sulfate was inhibited almost completely and more than 95 percent of the conjugates formed were found to be glucuronides.

Robinson, et al. (1951) studied the metabolic detoxification of the mononitrophenol isomers in rabbits. They showed that, with doses of 0.2 to 0.3 g/kg, conjugation in vitro with glucuronic and sulfuric acids was almost complete. Only small amounts (less than one percent) of the unchanged nitrophenol were excreted. With all three of the mononitrophenol isomers, the major conjugation product was nitrophenyl-glucuronide, which accounted for about 70 percent of the dose. The corresponding sulfate conjugates were

also excreted. Reduction of the nitrophenols occurred to a small extent, the reduction of the 4- isomer being slightly greater than that of the 2- and 3- isomers. The mononitrophenols were also shown to undergo oxidation to a very small extent (less than one percent). 2-Nitrophenol yields traces of nitroquinol; 3-nitrophenol yields nitroquinol and 4-nitrocatechol; and 4-nitrophenol yields 4-nitrocatechol.

A summary of the metabolism of the mononitrophenols is shown in Table 3. Data directly addressing the metabolic fate of the mononitrophenols in humans are not available. However, it is expected that following exposure to the mononitrophenols humans will rapidly excrete both glucuronide and sulfate conjugates in the urine.

Excretion

Data directly addressing the excretion of the mononitrophenols following exposure of humans were not found in the literature. However, excretion patterns for 4-nitrophenol following human exposure to parathion may shed some light on their elimination kinetics. Arterberry, et al. (1961) studied the pharmacodynamics of 4-nitrophenol excretion following exposure to parathion. They noted that the excretion of 4-nitrophenol in the urine was quite rapid "as might be expected in the case of a water-soluble metabolite of a substance which is quickly broken down by the animal organism." 4-Nitrophenol usually had disappeared from the urine within about 48 hours after cessation of exposure. In a similar study of orchard spraymen involved in the application of parathion, Wolfe, et al. (1970) noted that urinary levels of 4-nitrophenol rose promptly in response to parathion and returned to the nondetectable level after several days. Myslak, et al. (1971) reported on the excretion of 4-nitrophenol from a 19-year-old female subject following a suicidal oral dose of nitrobenzene. Large quantities of

TABLE 3
Urinary Metabolites of Mononitrophenols in Rabbits*

Nitrophenol	Nitro Compounds (N)	Amino Compounds (A)	Percentage of Dose Excreted as			
			(N + A) ¹	Glucuronides (G)	Ethereal Sulfates (E)	(G + E)
2-Nitrophenol	82	3	85	71	11	82
3-Nitrophenol	74	10	84	78	19	98
4-Nitrophenol	87	14	101	65	16	81

*Source: Robinson, et al. 1951.

¹(N + A) should be roughly equal to (G + E) since the amounts of free phenols excreted were very small. Both glucuronides and ethereal sulphates include nitro and amino conjugates.

4-nitrophenol and 4-aminophenol were detected in the urine. Elimination of 4-nitrophenol in the urine was expressed by the equation $V_t/V_0 = e^{-0.008t}$ where V_0 and V_t denote the excretion rate at the interval time 0 and t measured in hours. The half-life for excretion corresponded to about 84 hours.

Shafik, et al. (1973) studied the urinary excretion of 4-nitrophenol following administration of the pesticide EPN. Following oral administration of the pesticide for three days, animals were maintained and urine samples collected at 24-hour intervals. Three days were required for complete excretion of 4-nitrophenol under these conditions. The foregoing studies indicate that 4-nitrophenol is rapidly excreted following its production in vivo from other organic compounds.

Only one study was found that examined excretion of 4-nitrophenol following direct administration of the compound. Lawford, et al. (1954) studied the elimination of various nitrophenolic compounds from the blood of experimental animals. Elimination of 4-nitrophenol by the monkey following oral and intraperitoneal doses of 20 mg/kg body weight was complete within five hours. Elimination by mice, rats, rabbits, and guinea pigs was also rapid. Most doses were eliminated completely from the blood within two hours of administration. Experimental animals eliminated 4-nitrophenol from the blood in the following descending order of efficiency: mouse, rabbit, guinea pig, rat, and monkey.

In summary, the available data indicate that the mononitrophenols are excreted rapidly via the urinary route and that total elimination time is likely not to exceed one week. The mononitrophenols are highly water soluble and accumulation or bioconcentration in various tissues is not expected to occur to a large extent. However, much more data are needed to precisely define the transport distribution and elimination of these compounds in humans.

EFFECTS

Threshold concentrations for odor, taste, and color for 2-nitro-, 3-nitro-, and 4-nitrophenols in reservoir water have been reported in an abstract of a paper from the Russian literature (Makhinya, 1964). Reported threshold concentrations for 2-nitrophenol were 3.83 mg/l for odor, 8.6 mg/l for taste, and 0.6 mg/l for color. Concentrations for 4-nitrophenol were 58.3, 43.4, and 0.24 mg/l for odor, taste, and color, respectively. The values for 3-nitrophenol were given as 389, 164.5, and 26.3 mg/l. Acceptability thresholds from the standpoint of human consumption were not reported by these investigators.

Acute, Subacute, and Chronic Toxicity

Known effects of 4-nitrophenol demonstrated in animal experiments are methemoglobinemia, shortness of breath, and initial stimulation followed by progressive depression (von Oettingen, 1949).

Acute toxicity information for the mononitrophenol isomers has been compiled and presented as Table 4. 4-Nitrophenol is the most toxic of the mononitrophenols followed by 3-nitrophenol and 2-nitrophenol in relative toxicity. Toxicologic symptoms of mononitrophenol poisoning have not been well described in the literature. Sax (1968) noted that 2-nitrophenol exposure produced kidney and liver injury in experimental animals. Methemoglobin formation as a result of mononitrophenol exposure has also been reported (Patty, 1963). Grant (1959), however, was unable to detect methemoglobin formation after oral administration of 3-nitro- and 4-nitrophenols to rats. Small inconstant amounts of methemoglobin were formed after 3-nitrophenol administration. Smith, et al. (1967) were able to show that the reduction products of mononitrophenols, 2- and 4-aminophenol, would produce methemoglobin in female mice. Methemoglobin formation, therefore, may depend on

TABLE 4
Acute Toxicity of Mononitrophenol Isomers

Species	Dose (mg/kg)	Route of Administration	Effects	References
<u>2-Nitrophenol</u>				
Frog	300	s.c.	Lethal Dose	Spector, 1956
Mouse	600	i.m.	Lethal Dose	Spector, 1956
Rabbit	1700	s.c.	Lethal Dose	Spector, 1956
Cat	600	s.c.	Lethal Dose	Spector, 1956
Dog	100	i.v.	Lethal Dose	Spector, 1956
Rat	2830	Oral	LD50	Vernot, et al. 1977
Mouse	1300	Oral	LD50	Vernot, et al. 1977
Guinea Pig	900	s.c.	Lethal Dose	Spector, 1956
<u>3-Nitrophenol</u>				
Dog	83	i.v.	Minimum Lethal Dose	Spector, 1956
Rat	930	Oral	LD50	Vernot, et al. 1977
Mouse	1410	Oral	LD50	Vernot, et al. 1977
<u>4-Nitrophenol</u>				
Frog	50	s.c.	Minimum Lethal Dose	Spector, 1956
Rabbit	600	s.c.	Minimum Lethal Dose	Spector, 1956
Cat	197	s.c.	Minimum Lethal Dose	Spector, 1956
Dog	10	i.v.	Lethal Dose	Spector, 1956
Rat	620	Oral	LD50	Vernot, et al. 1977
Mouse	470	Oral	LD50	Vernot, et al. 1977
Rat	350	Oral	LD50	Fairchild, 1977

the capacity of the organism to reduce the mononitrophenols. As mentioned in the metabolism section of this document, reduction of the nitrophenols does not normally occur to any large extent.

Ogino and Yasukura (1957) reported the development of cataracts in vitamin C-deficient guinea pigs following administration of 4-nitrophenol. Cataracts developed in two of three guinea pigs on days 7 and 11 following daily intraperitoneal administration of 8.3 to 12.5 mg 4-nitrophenol/kg body weight. Subchronic administration of 4-nitrophenol over a 20-day test period produced cataracts while 2- and 3-nitrophenols did not. The authors concluded that the para-positioning of the hydroxyl- and nitro- groups is necessary for cataract induction.

Several deficiencies in this study preclude definitive conclusions on the cataractogenic properties of 4-nitrophenol. The investigators failed to report results on control animals, either totally untreated or treated with the nitrophenols and a vitamin C supplement. Thus, it is possible, based on the results reported, to conclude that vitamin C deficiency itself caused cataracts in some of the animals tested. The small size of the experimental groups (three animals/test compound) also make definitive conclusions difficult. The reported conclusions must be taken with considerable caution based on the above considerations.

In contrast, Dietrich and Beutner (1946) found 2-nitro- and 4-nitrophenols to be devoid of cataract-forming activity in seven-day-old chicks. Animals were fed on a commercial brand of chick food containing 0.25 percent nitrophenol. Although cataracts developed rapidly (within 24 to 48 hours) when the animals were fed 2,4-dinitrophenol, no cataracts developed within a three-week period when animals were fed the mononitrophenol isomers. The capacity for cataract formation in humans following mononitrophenol exposure is unclear.

Both 2-nitro- and 4-nitrophenol have been shown to inhibit porcine heart malate dehydrogenase in vitro (Wedding, et al. 1967). The compounds acted as competitive inhibitors for NAD in the forward direction of the enzymatic reaction. The clinical significance of these findings is unknown.

The ventilatory effects of the mononitrophenols have been examined in anesthetized rats (Grant, 1959). Test compounds were administered by stomach tube: 2-nitrophenol, 60 to 120 mg; 3-nitrophenol, 20 to 45 mg; 4-nitrophenol, 7 to 12 mg. Significant increases in respiratory volume ranging from 15 to 30 percent were reported in these experiments.

Neither carbon dioxide output nor oxygen uptake were affected by sublethal doses of 2-nitrophenol in rats (Cameron, 1958). In contrast, oxygen uptake was decreased in 3-nitrophenol-treated rats while carbon dioxide output was increased following 4-nitrophenol administration. Rectal temperature was depressed in rats receiving any of three isomers. These results suggest that mononitrophenol isomers are not potent uncouplers of oxidative phosphorylation, in contrast to the chemically similar compound 2,4-dinitrophenol.

Although the mechanism of toxic action of the mononitrophenols is not well understood, the following studies suggest that an action directly on cell membranes may occur. 3-Nitrophenol binds readily to red blood cell (RBC) membranes. Expansion of RBC ghosts occurs following nitrophenol treatments, as measured by the resistance of such ghosts to hemolysis (Machleidt, et al. 1972). 2-Nitrophenol and 4-nitrophenol inhibit chloride transport in red blood cells (a metabolism-independent process) suggesting a direct action on the cell membrane (Motais, et al. 1978). Further information on the acute or chronic toxicity of the mononitrophenols to humans was not found.

The National Institute for Occupational Safety and Health (NIOSH), recently undertook a health hazard evaluation determination at the request of an employee of the Boeing Company who had routinely handled 4-nitrophenol (Butler and Bodner, 1973). A 15 percent solution of 4-nitrophenol and methylphenol was painted on the exposed cork surfaces of the Minuteman Missile before arrival at the assembly plant. If the surface was damaged in transit it was necessary to apply small amounts of the 4-nitrophenol solution to the repaired areas of cork. The worker in question was engaged in such touch-up operations. Workers routinely wore an organic vapor cartridge respirator, a face shield, cotton gloves, rubber gloves, and were completely covered with protective clothing. The employee complained of fatigue, joint pain, abdominal cramps and diarrhea, and attributed these symptoms to his exposure to both the treating solution and the dried cork impregnated with 4-nitrophenol during his work as a mechanic. Medical examination failed to detect 4-nitrophenol in the urine but revealed a complete absence of the immunoglobins IgA and IgD in the employee. Based on medical judgement and the existing literature, the study concluded that the employee's condition stemmed from the lack of IgA and IgD and that this deficiency was not caused by exposure to 4-nitrophenol.

Gabor, et al. (1960) reported a unique effect of 2-nitrophenol on blood platelet levels. When 31 rats were administered 2-nitrophenol by intraperitoneal injection at 1 mg/kg body weight, the platelet count increased significantly. Even at doses of 0.1 mg/kg a similar effect was produced. Administration of 3-nitro or 4-nitrophenol did not produce a rise in platelet levels. Additional data are not available to explain this unique phenomenon, nor is the clinical significance of these findings known.

A report from the Russian literature (Makhinya, 1969) reports that 2-nitro-, 3-nitro-, and 4-nitrophenols possess distinct cumulative properties. Chronic administration of any of the mononitrophenols to mammals caused alterations of neurohumoral regulation and pathological changes including colitis, enteritis, hepatitis, gastritis, hyperplasia of the spleen, and neuritis. Limiting doses for the disruption of conditioned reflex activity were established as 0.003 mg/kg (equivalent to 0.006 mg/l of water) for 2-nitrophenol and 3-nitrophenol and 0.00125 mg/kg (equivalent to 0.0025 mg/l of water) for 4-nitrophenol. Unfortunately a report of this study was available in abstract form only. Details of the experiment including animal species, mode of administration, duration of the treatment, and a complete description of the observed biological effects, were not reported. The results must be considered questionable until evaluation of the experimental protocol is possible.

Synergism and/or Antagonism

Only one report was found dealing with possible synergistic effects of the mononitrophenols. Cairns, et al. (1976) studied the effects of a sublethal exposure to zinc and subsequent toxicity of 4-nitrophenol to snails, Goniobasis livescens. Snails were exposed for 96 hours to two sublethal concentrations of zinc (1.54 mg/l and 3.08 mg/l corresponding to 0.2 and 0.4 of the 48-hour LC₅₀ dose, respectively) followed by an acutely lethal dose of 4-nitrophenol (1,000 mg/l). A significantly reduced survival time following exposure to 4-nitrophenol was reported. In a second experiment, snails were exposed to sublethal levels of 4-nitrophenol (13.2 mg/l) and subjected to a lethal temperature shock 96 hours later. A significant decrease in the median survival time of the snails during the temperature shock was noted. The applicability of these data to humans or mammals is unknown. Data regarding synergistic or antagonistic effects of the mononitrophenols in mammals were not found.

Teratogenicity

Pertinent information could not be located in the available literature regarding teratogenic properties of the mononitrophenols.

Mutagenicity

Szybolski (1958) tested the three mononitrophenol isomers for their ability to induce streptomycin-independence in streptomycin-independent E. coli. All three isomers gave negative results.

Buselmaier, et al. (1976) tested 4-nitrophenol for mutagenic activity in mice with the host mediated assay and the dominant lethal method, using Salmonella typhimurium G46 His⁻, Serratia marcescens a21 Leu⁻, and Serratia marcescens a31 His⁻, as indicator organisms. Spot tests in vitro were also performed. Mutagenic activity was not demonstrated.

4-Nitrophenol also failed to induce mutations in Salmonella both with and without microsomal activation (McCann, et al. 1975).

Fahrig (1974) demonstrated a weak mutagenic activity when 4-nitrophenol was tested for mitotic gene conversion in Saccharomyces cerevisiae. This test system allows the detection of a genetic alteration whose molecular mechanism is presumably based on the formation of single-strand breaks of DNA.

Adler, et al. (1976) used the difference in growth inhibition of wild type Proteus mirabilis and the corresponding repair-deficient strain as an indication of DNA damage. 4-Nitrophenol showed some evidence of DNA damage in this system.

Effects on mitosis and chromosome fragmentation have been reported in plants. Sharma and Ghosh (1965) examined the mitotic effects of the mononitrophenol isomers in root tips of Allium cepa. Inhibition of mitosis in root tips was reported for all three mononitrophenol isomers but only 4-nitrophenol induced detectable chromosome fragmentations. Amer and Ali

(1969) studied the effects of 2- and 4-nitrophenols on the lateral root mitoses of Vicia faba seedlings. The mitotic index was reduced at concentrations of these compounds ranging from 0.025 percent to 0.1 percent. Induction of anaphase bridges by both isomers was noted but (in agreement with the work of Levin and Tjio (1948) with Allium cepa) chromosome fragmentation was not detected. The relationship of these changes in plants to alterations in mammalian cells has not been established. Based on the available data, the mononitrophenols do not appear to pose a mutagenic hazard to humans.

Carcinogenicity

Data on the possible carcinogenicity of the mononitrophenols are scant in the literature. Boutwell and Bosch (1959) have studied the ability of a number of phenolic compounds to promote tumor formation on mouse skin following a single initiating dose of dimethylbenzanthracene. Although phenol itself has demonstrated a promoting capacity in this system, both 2- and 4-nitrophenols failed to promote tumor development in mice. No other data on possible carcinogenic potential of the mononitrophenols were found.

4-Nitrophenol has been selected by the National Cancer Institute (NCI) for testing under the Carcinogenesis Bioassay Program.

DINITROPHENOLS

Mammalian Toxicology and Human Health Effects

INTRODUCTION

Six isomeric forms of dinitrophenol are possible, distinguished by the position of the nitro-groups on the phenolic ring. Of the six possible dinitrophenol isomers, 2,4-dinitrophenol is by far the most important. The most recent production figure for 2,4-dinitrophenol is 863,000 lbs. reported by the U.S. International Trade Commission (1968). Approximate consumption per year is estimated at 1,000,000 lbs. (Howard, et al. 1976). 2,4-Dinitrophenol is used primarily as a chemical intermediate for the production of sulfur dyes, azo dyes, photochemicals, pest control agents, wood preservatives, and explosives (Matsuguma, 1967; Perkins, 1919; Springer, et al. 1977a,b).

Production figures and usage data for the remaining five dinitrophenol isomers are not available. It is reasonable to assume that production and usage of these compounds are extremely limited in the United States.

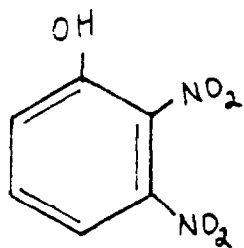
Commercial synthesis of 2,4-dinitrophenol is accomplished by the hydrolysis of 2,4-dinitro-1-chlorobenzene with sodium hydroxide at 95 to 100°C (Matsuguma, 1967). As a result of the use pattern of 2,4-dinitrophenol (2,4-DNP) the major source for environmental release of 2,4-DNP is likely to be from production plants and chemical firms where the compound is used as an intermediate. It is possible that 2,4-DNP may also be produced via microbial or photodegradation of compounds which contain the nitrophenol moiety, such as parathion (Gomaa and Faust, 1972). 2,4-DNP has also been identified as a impurity in technical preparations of the herbicide DNP (2-isopropyl-4,6-dinitrophenol) by Mosinska and Kotarski (1972).

The physical and chemical properties of the dinitrophenol isomers are summarized in Table 5 and Figure 2.

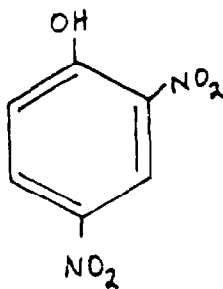
TABLE 5
Properties of Dinitrophenol Isomers*

Isomer	m.p. (°C)	K (at 25°C)	Water Solubility (g/l)	Density
2,3-Dinitrophenol	144	1.3×10^{-5}	2.2	1.681
2,4-Dinitrophenol	114-115 (sublimes)	1.0×10^{-4}	0.79	1.683
2,5-Dinitrophenol	104	7×10^{-6}	0.68	
2,6-Dinitrophenol	63.5	2.7×10^{-4}	0.42	
3,4-Dinitrophenol	134	4.3×10^{-5}	2.3	1.672
3,5-Dinitrophenol	122-123	2.1×10^{-4}	1.6	1.702

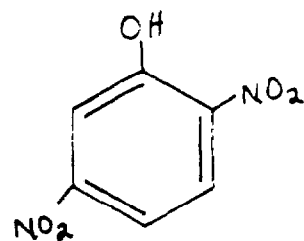
*Source: Harvey, 1959; Windholz, 1976; Weast, 1975.



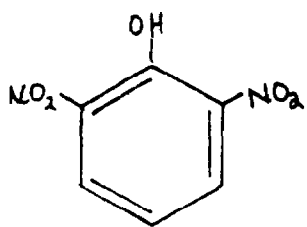
2,3-dinitrophenol



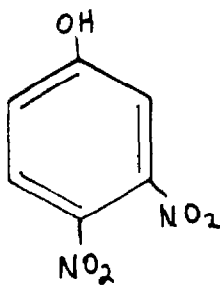
2,4-dinitrophenol



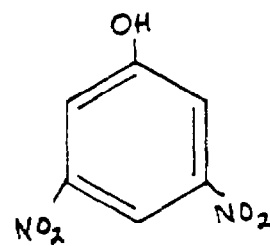
2,5-dinitrophenol



2,6-dinitrophenol



3,4-dinitrophenol



3,5-dinitrophenol

FIGURE 2
Dinitrophenols

EXPOSURE

Ingestion from Water

No data were available regarding human exposure via ingestion of dinitrophenols from water.

The enhancement of biological waste water treatment by 2,4-DNP has been examined (Shah, 1975; Shah, et al. 1975). Addition of 0.92 mg/l 2,4-DNP to waste water systems results in an increase of 85 percent in waste degrading rate and a decrease of 70 percent in cell growth. Shah, et al. (1975) note that the optimum concentration for 2,4-DNP in this system (0.92 mg/l) is undesirably high from the standpoint of current Federal effluent regulations but that the compound is completely eliminated by adsorption on activated carbon which generally follows biological treatment of waste waters. It is not known whether this treatment method is currently used in the United States. Theoretically, such usage might result in 2,4-DNP contamination of surface waters.

Games and Hites (1977) detected dinitrophenol (isomer not identified) in the effluent waters of a dye manufacturing plant. Dinitrophenol at 300 to 400 $\mu\text{g/l}$ was detected in raw waste water, prior to biological treatment. The final plant effluent contained dinitrophenol at 42 to 270 $\mu\text{g/l}$. Mud and river water samples downstream from the effluent point were analyzed by gas chromatography/mass spectrometry. Dinitrophenol was not detected in these samples.

The persistence of dinitrophenol isomers in ambient waters has not been well studied. A number of investigators have studied the bacterial degradation of the dinitrophenols utilizing acclimated populations of microorganisms. Phenol-adapted bacteria obtained from garden soil, compost, and river

mud degraded 2,4-dinitrophenol in seven to ten days (Tabak, et al. 1964). 2,6-Dinitrophenol was degraded very slowly in this system. 2,4-, 2,5-, and 2,6-Dinitrophenols were tested for biological degradability by an activated sludge culture obtained from a sewage treatment plant (Pitter, 1976). 2,5-Dinitro- and 2,6-dinitrophenols were not degraded in this system although 85 percent removal of 2,4-dinitrophenol was achieved within 20 days. Further degradation of 2,4-dinitrophenol did not occur in this system, however. Bacteria isolated from parathion-treated flooded soil (Sudhakar-Barik, et al. 1976) degraded 2,4-dinitrophenol after an exceptionally long lag period. Nitrite was produced only in trace amounts after 25 days. Even after 50 days, only eight percent nitrogen was accounted for as nitrite.

The available data indicate that dinitrophenols are susceptible to partial degradation by certain microorganisms. Of the dinitrophenol isomers, 2,4-DNP appears to be most easily degraded. It may be speculated that dinitrophenols will be subject to microbial attack in environmental situations where acclimated microbiological populations exist (e.g., sewage treatment plants). The persistence of dinitrophenols in the environment where acclimated microbial populations do not exist is speculative.

Ingestion from Food

Pertinent data could not be located in the available literature regarding exposure to dinitrophenols via ingestion of food.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenols, but the equation " $\text{Log BCF} = (0.85 \text{ Log } P) - 0.70$ " can be used (Veith, et al. 1979) to estimate the steady-state BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). The log P values were obtained from Hansch and Leo (1979) or were calculated by the method described therein.

The adjustment factor of $3.0/7.6 = 0.395$ is used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish in order to obtain the weighted average bioconcentration factor for the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans.

Inhalation

Dinitrophenol isomers may be produced in the atmosphere through a photochemical reaction between benzene and nitrogen monoxide. Nojima, et al. (1975) irradiated a combination of benzene vapor and nitrogen monoxide for five hours with a xenon lamp and characterized the following resulting photochemical products: nitrobenzene, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, and 2,6-dinitrophenol. The authors suggested that these nitro-compounds may be the cause of the characteristic symptoms of seriously stricken victims of photochemical smog in Japan, which include headache, breathing difficulties, vomiting, rise in body temperature and numbness in the extremities. In the absence of monitoring data it is impossible to estimate the extent of human exposure to dinitrophenols as a result of their photochemical production in the atmosphere.

Dermal

2,4-DNP is rapidly absorbed through the intact skin (Gleason, et al. 1969). Although no direct information on the other dinitrophenol isomers is available, it is reasonable to suppose that dermal absorption will readily occur with these compounds as well. Since 2,4-DNP is used primarily as a chemical intermediate, dermal exposure is expected to occur most often in an industrial setting. 2,4-DNP is also used occasionally as a spray against aphids and mites, as a fungicide for certain molds and mildews, as a weed

killer, and as an ingredient in some wood preservative formulations (Gleason, et al. 1969). Dermal exposure to humans may occur among individuals handling 2,4-DNP in these applications. Data on the importance of the dermal exposure route of dinitrophenols in humans are not available.

PHARMACOKINETICS

Absorption

Absorption of dinitrophenol (isomer unspecified) through the skin and following inhalation occurs readily (von Oettingen, 1949).

Gehring and Buerge (1969b) reported that 2,4-DNP is absorbed very rapidly by ducklings and rabbits following intraperitoneal administration. In fact, immature rabbits absorbed the administered DNP so rapidly that an absorption constant could not be calculated from the data. DNP concentration in serum peaked within five minutes of administration.

Other quantitative information on the rate of absorption of the dinitrophenol isomers was not found.

Distribution

Blood levels of the dinitrophenols rise rapidly following absorption (Gehring and Buerge, 1969b; Harvey, 1959) suggesting that the dinitrophenol isomers are transported by the blood regardless of the mode of absorption. 2,4-DNP binds to serum proteins following intraperitoneal administration to rabbits and ducklings. Early after the administration of 2,4-DNP, the concentration of free DNP in serum is much greater than the bound form, and at later times the reverse is true (Gehring and Buerge, 1969b).

Based on the available data, the dinitrophenol isomers do not appear to be stored to any significant extent in the tissues of human or experimental animals following absorption. Gisclard and Woodward (1946) unsuccessfully attempted to extract 2,4-dinitrophenol or its metabolites from the tissues of two human victims of fatal intoxication.

It seems likely, based on the short half-lives of these compounds in mammals, that the large majority of any dose will be rapidly excreted via the urine. On the other hand, von Oettingen (1949) reported both dinitrophenol (unspecified isomer) and aminonitrophenol in the liver, kidney, brain, blood, and spinal fluid of dogs after fatal doses of dinitrophenol. Recent work on the tissue distribution of the dinitrophenols following absorption in mammals was not found.

Metabolism

In a study of the munitions industry in France (Perkins, 1919) it was reported that the urine of men fatally poisoned by 2,4-DNP contained: 2-amino-4-nitrophenol, 4-amino-2-nitrophenol, diamino-phenol, and a number of nitrogen compounds resulting from a combination of two molecules of aminonitrophenol or of diaminophenol. It has frequently been reported that 2-amino-4-nitrophenol invariably exists in the urine of persons suffering from serious intoxication by 2,4-DNP. Williams (1959) stated that 2,4-DNP is excreted in mammals in the following forms: partially unchanged; partially conjugated with glucuronic acid; reduced to 2-amino-4-nitrophenol, 2-nitro-4-aminophenol and probably 2,4-diaminophenol. Rats orally dosed with 2,4-DNP at 1.5 to 12 mg/kg excreted both free dinitrophenol (78 percent) and 2-amino-4-nitrophenol (17 percent) (Senzuk, et al. 1971).

Although the in vitro metabolism of 2,4-dinitrophenol has not been extensively studied in mammalian systems, Parker (1952) examined the enzymatic reduction of 2,4-DNP by rat liver homogenates and found 4-amino-2-nitrophenol to be the major metabolite. The metabolite 2-amino-4-nitrophenol comprised less than 10 percent of the total metabolites formed; 2,4-diaminophenol was found in trace amounts. Presumably the latter metabolite was formed from the reduction of the remaining nitro- group of one of the two above compounds.

In contrast, Eiseman, et al. (1974) reported that 2-amino-4-nitrophenol was the major metabolite (75 percent of total amino). In the latter study 4-amino-2-nitrophenol was formed in considerably smaller amounts (23 percent) when 2,4-DNP was enzymatically reduced in vitro by rat liver homogenates. These investigators also detected only traces of diaminophenol indicating that it may be a secondary reduction product as suggested by Parker (1952). A precise definition of the metabolic fate of the dinitrophenols in humans awaits further investigation.

Excretion

Data on the elimination kinetics of the dinitrophenols or their metabolic products in humans were not found. Edsall (1934) stated: "Judging from the metabolic response, DNP appears to be eliminated entirely in three or four days; in the presence of liver or kidney damage it is possible that the drug will be retained over a longer period." Information on the elimination kinetics of the dinitrophenols from experimental animals is also scant in the literature.

Genring and Buerge (1969b) have developed equations which describe the elimination of 2,4-DNP from the serum of ducklings, mature rabbits, and immature rabbits following intraperitoneal administration of the compound. Serum levels of 2,4-DNP in the mature rabbit declined to less than one percent of their original high values within seven hours. Twenty-four hours were required before the serum levels in the immature rabbit declined to two percent of their original values. Ducklings eliminated 2,4-DNP from the serum over a similar time frame (96 percent elimination in 24 hours).

Lawford, et al. (1954) also studied the elimination of various nitrophenolic compounds (including 2,4-dinitrophenol). Elimination from the blood of mice, rabbits, guinea pigs, rats, and monkeys was complete within

30 hours. Harvey (1959) calculated the elimination rates of all six dinitrophenol isomers from the blood of mice and rats following a single large dose given intraperitoneally. Data are presented in Table 6. The data developed by these investigators must be taken with caution since the actual elimination of the dinitrophenols or their metabolites in urine was not directly measured. In view of the lack of data suggesting concentration of the dinitrophenols in mammalian tissues and the high water solubility of these compounds, their elimination via the urine may be a rapid process in humans.

EFFECTS

Acute, Subacute, and Chronic Toxicity

All of the dinitrophenol isomers are potent metabolic poisons. Most of the literature available deals with 2,4-dinitrophenol since this compound has been used extensively for more than 70 years. A number of excellent reviews on the uses, chemistry, mode of action, and mammalian toxicity of 2,4-dinitrophenol are available (Edsall, 1934; Metcalf, 1955; Horner, 1942; Simon, 1953; Slater, 1962; Parascandola, 1974; Howard, et al. 1976) and no attempt will be made to duplicate the information found in these documents.

2,4-Dinitrophenol is considered a classic uncoupler of oxidative phosphorylation and is widely used by biochemists to determine whether a given biochemical process is energy dependent. Hence, an enormous body of literature has been generated dealing with the biochemical effects of 2,4-dinitrophenol on cellular and biochemical processes both in vivo and in vitro. Only those studies with direct relevance to the acute or chronic effects of the dinitrophenols on humans are reviewed in this document.

The toxic action of the dinitrophenols is generally attributed to their ability to uncouple oxidative phosphorylation. These compounds prevent the

TABLE 6

Elimination Rates of Dinitrophenol Isomers from the Blood of Mice
and Rats Following a Single Large Intraperitoneal Dose*

Isomer	Dose (mg/kg)	Half-time for Elimination (min.)
MICE		
2,3-Dinitrophenol	90	2.7
2,4-Dinitrophenol	20	54.0
2,5-Dinitrophenol	180	3.3
2,6-Dinitrophenol	30	238.0
3,4-Dinitrophenol	60	3.5
3,5-Dinitrophenol	30	2.7
RATS		
2,3-Dinitrophenol	90	12.5
2,4-Dinitrophenol	20	225.0
2,5-Dinitrophenol	90	13.0
2,6-Dinitrophenol	25	210.0
3,4-Dinitrophenol	90	11.5
3,5-Dinitrophenol	30	2.1

*Source: Harvey, 1959.

utilization of the energy provided by cellular respiration and glycolysis by inhibiting the formation of high energy phosphate bonds. All energy dependent biochemical processes are therefore affected by the action of the compounds (Metcalf, 1955). The large number of clinical effects attributed to dinitrophenol toxicity result essentially from the shortcircuiting of metabolism in cells which absorb sufficient dinitrophenol.

All six dinitrophenol isomers are potent uncouplers of oxidative phosphorylation. The relative potencies of the six dinitrophenols in uncoupling phosphorylation in rat liver mitochondria were found to be (in declining order): 3,5- → 2,4- → 2,6- = 3,4- → 2,3- = 2,5-dinitrophenol (Burke and Whitehouse, 1967). 3,5-Dinitrophenol is approximately five times more potent than 2,5-dinitrophenol as measured in this system. The relative in vivo toxicities of the dinitrophenol isomers have been determined by a number of investigators (von Oettingen, 1949; Harvey, 1959; Cameron, 1958; Grant, 1959; Levine, 1977) and the order of relative potency of the isomers determined in these investigations frequently differs from the order developed by Burke and Whitehouse (1967). Several explanations for these discrepancies are possible: (1) differential tissue absorption of the isomers or (2) different metabolic detoxification mechanisms for the isomers or (3) the presence of cellular or biochemical effects unrelated to the uncoupling of oxidative phosphorylation. Resolution of this question awaits further investigation.

At concentrations higher than those necessary to uncouple oxidative phosphorylation, a number of inhibitory effects of the dinitrophenol isomers on certain enzymatic reactions may occur.

Both 2,4-dinitro- and 3,5-dinitrophenol inhibit porcine heart malate dehydrogenase in vitro (Wedding, et al. 1967). Inhibition of the reaction

occurred at nitrophenol concentrations 10 to 100 times those causing uncoupling, and resulted from a competitive inhibition with NAD in the forward direction of the malate dehydrogenase reaction. In a similar study Stockdale and Selwyn (1971) reported in the in vitro inhibition of both lactate dehydrogenase and hexokinase by 2,4-dinitro-, 2,5-dinitro-, 2,6-dinitrophenols.

The dinitrophenols may also act directly on the cell membrane, thus causing toxic effects on cells which do not depend on oxidative phosphorylation for their energy requirements. 2,4-Dinitro-, 2,5-dinitro-, and 2,6-dinitrophenols inhibit passive permeability to chloride (a metabolically independent process) in red blood cells (Métais, et al. 1978).

Acute toxicity information for the dinitrophenols has been compiled and presented in Table 7.

Numerous occasions of human poisoning by 2,4-DNP have been reported in the literature. The earliest cases of fatal 2,4-DNP intoxication relate to its usage as a component of explosives during World War I. Thirty-six cases of fatal occupational dinitrophenol poisoning occurred among employees of the munitions industry in France between 1916 and 1918 (Perkins, 1919). A literature review by von Oettingen (1949) revealed 27 reported cases of fatal occupational dinitrophenol poisoning in the United States for the years 1914 to 1916.

Gisclard and Woodward (1946) reported two fatal cases of dinitrophenol poisoning during manufacture of picric acid where 2,4-DNP was produced as an intermediate. Swamy (1953) describes a case of suicidal poisoning by 2,4-DNP.

Early in the 1930s, 2,4-dinitrophenol was widely recommended as a treatment for obesity. Dinitrophenol was received with overwhelming popularity

TABLE 7

Acute Toxicity of Dinitrophenol Isomers

Species	Dose (mg/kg)	Route of Administration	Effects	References
<u>2,4-Dinitrophenol</u>				
Rat	25	s.c.	LD50	von Oettingen, 1949
Rat	35	i.p.	LD50	Harvey, 1959
Rat	30	Oral	LD50	Spector, 1956
Rat	28.5	i.p.	LD50	Lawford, et al. 1954
Rat	31	i.p.	LD100	Gatz and Jones, 1970
Mouse	36	i.p.	LD50	Harvey, 1959
Mouse	26	i.p.	LD50	Lawford, et al. 1954
Guinea Pig	700	Dermal	Lethal Dose	Soencer, et al. 1948
Rabbit	30	s.c.	LD50	von Oettingen, 1949
Rabbit	200	Oral	LD50	Spector, 1956
Rabbit	100	i.p.	Lethal Dose	Spector, 1956
Dog	30	UNK	MLD	Harvey, 1959
Dog	20-30	Oral	LD50	Spector, 1956
Dog	22	s.c.	LD50	Spector, 1956
Dog	20	i.m.	LD50	Spector, 1956
Dog	30	i.v.	LD50	Spector, 1956
Pigeon	7	i.m.	Lethal Dose	Spector, 1956
Pigeon	15-20	i.v.	Lethal Dose	Spector, 1956
Human	40 mg/m ³	Inhalation	Lethal Conc.	MacBryde and Taussig, 1935
Human	1-3 g	Oral	Lethal Dose	Sax, 1968
Human	4.3	Oral	Lethal Dose	Geiger, 1933
<u>2,3-Dinitrophenol</u>				
Rat	190	i.p.	LD50	Harvey, 1959
Mouse	200	i.p.	LD50	Harvey, 1959
Dog	1000	UNK	MLD	Harvey, 1959

TABLE 7 (continued)
Acute Toxicity of Dinitrophenol Isomers

Species	Dose (mg/kg)	Route of Administration	Effects	References
<u>2,5-Dinitrophenol</u>				
Rat	150	i.p.	LD50	Harvey, 1959
Mouse	273	i.p.	LD50	Harvey, 1959
Dog	100	UNK	MLD	Harvey, 1959
<u>2,6-Dinitrophenol</u>				
Rat	38	i.p.	LD50	Harvey, 1959
Mouse	45	i.p.	LD50	Harvey, 1959
Dog	50	UNK	MLD	Harvey, 1959
<u>3,4-Dinitrophenol</u>				
Rat	98	i.p.	LD50	Harvey, 1959
Mouse	112	i.p.	LD50	Harvey, 1959
Dog	500	UNK	MLD	Harvey, 1959
<u>3,5-Dinitrophenol</u>				
Rat	45	i.p.	LD50	Harvey, 1959
Mouse	50	i.p.	LD50	Harvey, 1959
Dog	500	UNK	MLD	Harvey, 1959

s.c. = subcutaneous
i.p. = intraperitoneal
i.m. = intramuscular
i.v. = intravenous
UNK = unknown
MLD = minimum lethal dose

(Horner, 1942) as a slimming agent in spite of warnings of harmful side effects caused by disruption of the metabolic rate. It was estimated that during the first 15 months following its introduction, 100,000 persons took the drug for weight reduction (Horner, 1942). More than 1,200,000 capsules of 0.1 g each were dispensed from a single clinic in San Francisco. More than 20 drug houses offered to supply both dinitrophenol and mixtures containing the drug. Many of these remedies could be procured without prescription and with no further directions than to take "one capsule three times daily after meals." In view of this widespread and uncontrolled usage of the compound, it is not surprising that both toxic side effects and fatalities resulted. Horner (1942) reported a total of nine deaths resulting from the use of dinitrophenol as a slimming agent.

Parascandola (1974) reviewed the history and public concern which developed over dinitrophenol in the United States. An article appearing in Newsweek (1933) entitled "Diet and Die with Excess Alpha Dinitrophenol" was typical of public concern generated by misuse of dinitrophenol. In the wake of reports that cataract development in humans attributable to dinitrophenol was occurring, the drug was finally withdrawn from use in 1937.

The toxic manifestations of dinitrophenol exposure as reviewed by Horner (1942), included subacute symptoms such as gastrointestinal disturbances (nausea, vomiting, colic, diarrhea, anorexia), profuse sweating, weakness, dizziness, headache, and loss of weight. Acute poisoning has resulted in the sudden onset of pallor, burning thirst, agitation, dyspnea, profuse sweating, and hyperpyrexia. Intense and rapid onset of rigor mortis after death has also been described. A physician who ingested a fatal overdose of dinitrophenol (estimated at 2.5 to 5 g) was literally "cooked to death" (Geiger, 1933). Rectal temperature at death exceeded 110°F.

Perkins (1919) made the interesting observation that postmortem examination of dinitrophenol victims demonstrated no characteristic lesions. Acute edema of the lungs was mentioned but was believed to be secondary to the toxic effects on the vasomotor system. Microscopic lesions of the liver and kidney cells were inconstant and typical changes were lacking elsewhere.

Spencer, et al. (1948) studied the chronic toxicity of 2,4-dinitrophenol in rats. Male rats were fed diets containing 0.01, 0.02, 0.05, 0.10, or 0.20 g of 2,4-dinitrophenol per 100 g of food. Rats were maintained on diets containing 2,4-dinitrophenol for six months and both hematological and pathological investigations on surviving animals were performed. Hematological examination included erythrocyte counts, hemoglobin concentrations, leukocyte counts, differential counts, and bone marrow counts at autopsy. Both gross and microscopic examination of liver, kidney, spleen, lung, heart, adrenal, pancreas, and stomach tissues were also performed. Rats maintained on diets containing 0.02 percent 2,4-DNP (corresponding to 5.4 to 20 mg/kg body weight/day) grew at a normal rate and the investigators failed to detect discernible ill effects or pathological changes at autopsy. Similarly, pathological changes were not found upon microscopic examination of tissues from rats receiving diets containing 0.05 percent 2,4-DNP (corresponding to 13.5 to 50 mg/kg/day) although growth of these rats fell five to ten percent below that of the controls throughout the six-month experimental period. At autopsy the only changes observed in these animals were a very slight depletion of body fat and a very slight increase in the average weight of the kidneys. At higher doses of 2,4-dinitrophenol in their diets (54 to 200 mg/kg body weight/day) rats occasionally died and survivors lost weight rapidly. Examination of surviving animals revealed marked emaciation, an empty gastrointestinal tract, a slightly enlarged and dark spleen,

and undersized testes. Microscopic examination showed slight congestion and cloudy swelling of the liver, very slight parenchymatous degeneration of the epithelium of the renal tubules, slight congestion and hemosiderosis of the spleen and testicular atrophy. No significant pathological changes were observed in the lung, heart, adrenals, pancreas, or stomach of these animals. Based on the work of Spencer, et al. (1948), a no-observable-effect-level for 2,4-DNP in rats lies between 5.4 and 20 mg/kg body weight/day.

Information on the subacute or chronic effects of the other dinitrophenol isomers in experimental animals was not found. Langerspectz and Tarkkonen (1961) failed to detect histological changes in the adrenals or the liver during 2,4-dinitrophenol treatment of Swiss albino male mice. 2,4-Dinitrophenol was administered via the subcutaneous injection of 10 mg of 2,4-DNP/kg twice daily for 30 days.

Arnold, et al. (1976) examined the effects on the kidney of a single large dose of 2,4-DNP. Although a dose close to the LD₅₀ was chosen, (20 mg/kg) only small areas of cortical tubular necrosis were observed in a few of the rats treated.

Tainter and Cutting (1933) administered 2,4-DNP to dogs at intervals of three or more days over a period of two to three months. Abnormal liver and kidney pathology were not detected but an effect on spleen tissue was noted. Over large areas of the material containing "numerous large faintly staining cells with vesicular polyhedral nuclei."

The widespread use of 2,4-dinitrophenol as a weight reducing agent in humans during the 1930s provides some information regarding the chronic effects of this compound in man. Recommended therapeutic doses of 2,4-DNP for weight control on humans ranged from 2 to 5 mg/kg body weight/day (Dunlop, 1934; Horner, 1942; Tainter, et al. 1933). Tainter, et al. (1933) administered 2,4-DNP to 113 obese patients for as long as four months without

demonstrating evidence of cumulative or toxic effects. The most important side effect noted by the investigator was a skin rash observed in about 7 percent of the patients treated. The rash was manifested usually after a one-day period of mild itching and consisted of a maculopapular or urticarial type of rash. The itching was intense and in some cases there was considerable swelling. Symptoms subsided in two to five days following withdrawal from the drug. The next most important side effect noted by the authors was a loss of taste for salt and sweets observed in 5.3 percent of the patients. This effect also subsided following withdrawal from 2,4-DNP. The investigators failed to detect any effect of 2,4-DNP on liver or kidney function, pulse, blood pressure, or oxygen capacity of the blood. No cases of anemia, agranulocytosis, or malignant neutropenia appeared. Three cases of mild gastrointestinal upset were reported, however.

The development of cataracts following dinitrophenol therapy was first described by Horner, et al. (1936). In a later publication, Horner (1942) reviewed the acute and chronic toxicity of use of 2,4-DNP (including cataract formation) resulting from therapeutic use of the compound. Gastrointestinal symptoms consisting of nausea, vomiting, and loss of appetite were common as a result of 2,4-DNP administration. Cutaneous lesions were the most frequent side effect with an incidence of 8 to 23 percent. Although the majority of lesions were mild, others were severe. Bone marrow effects of dinitrophenol have also been reported. Eight cases of agranulocytosis were reported, with three fatalities. Thirty cases of neuritis including aberrations of taste and multiple regional involvement, particularly affecting the feet and legs were recorded. Symptoms appeared after an average of ten weeks, followed ordinary therapeutic doses and persisted for weeks or

months. Electrocardiographic evidence of functional heart damage was offered by several investigators and fragmentation of the heart muscle was reported at autopsy in one fatal case. It was generally agreed that 2,4-DNP was rarely injurious to the liver and kidneys when administered in therapeutic doses.

Over 100 cases of cataract formation following dinitrophenol therapy were reviewed by Horner (1942). Horner described the following characteristic features of 2,4-DNP induced cataracts: (1) they occurred in young women who were physically normal save for varying degrees of obesity and were in an age group in which senile cataracts do not occur; (2) they were bilateral and appeared either during or after periods of dinitrophenol treatment; (3) an interval of months or years might elapse between the time the last dose was taken and the onset of blurred vision; (4) lenticular changes were strikingly similar and could be demonstrated with the biomicroscope at a time when vision for distance and reading was still normal; (5) after gradual onset, the lenticular changes progressed with startling rapidity until the vision was obscured; (6) treatment was without effect in staying their progress; and (7) surgical removal of the lens was uniformly successful in restoring vision.

The length of time that 2,4-DNP was taken and the amount of the drug consumed varied widely among cataract victims. In 29 cases, the duration of treatment varied from 3 months to 24 months with an average of 11 months. Neither the length of treatment nor the total dose seemed to have any bearing on the occurrence of cataracts. Individual susceptibility appeared to be a more important factor. Horner (1942) estimated that the incidence of cataracts in patients who had taken dinitrophenol exceed one percent.

Formation of cataracts by acute exposure to DNP was first demonstrated in animals almost ten years after the problem was known to exist in humans (Gehring and Buerge, 1969a; Ogino and Yasukura, 1957; Feldman, et al. 1959, 1960; Bettman, 1946). Experimental cataracts, first produced in ducks and chickens, differ from DNP-induced human cataracts in that they can be formed in acute exposures and may appear in less than one hour. Furthermore, these lesions will disappear spontaneously in animals with 25 hours (Howard, et al. 1976). Hence, the usefulness of data on the formation of cataracts in experimental animals following DNP administration in assessing human hazard to dinitrophenol is questionable.

The available data do not allow the calculation of a minimum effect level for 2,4-DNP-induced cataract formation in man. Cataractogenic activity in humans has been observed in a small proportion of patients receiving as little as 2 mg/kg body weight/day. An assessment of the no-effect-level for cataract formation awaits further investigation. Such an assessment is further complicated by the fact that cataract formation in humans, following DNP administration, differs significantly from the situation seen in experimental animal studies.

Synergism and/or Antagonism

A report of teratogenic synergism following the combined administration of 2,4-dinitrophenol and insulin to chicks was made by Landauer and Clark (1964). The injection of 2,4-dinitrophenol at 100 µg/egg was nontoxic and nonteratogenic after 96 hours of incubation. However, the combined administration of insulin (a known teratogen) with 100 µg of 2,4-dinitrophenol raised the incidence of embryo mortality from 16 to 19 percent and shortened the upper beak by 1.4 to 18.5 percent.

Both thyroid hormones and 2,4-dinitrophenol decrease the efficiency of mitochondrial oxidative phosphorylation in vivo and in vitro. The in vivo administration of both l-thyroxine and 2,4-DNP results in larger changes in metabolic rate and body temperature than are accounted for by the sum of the separate effects of each agent (Hoch, 1965).

Other direct information on possible synergism between the dinitrophenols and other chemical compounds is not available.

Teratogenicity

Wulff, et al. (1935) examined the effects of 2,4-dinitrophenol on the fertility, gestation, and fetal life of rats. They administered 20 mg of 2,4-DNP/kg body weight to female rats eight days prior to the introduction of males. Dinitrophenol was administered intragastrically twice daily until the respective litters were weaned. The average number born in each litter was not affected by the use of dinitrophenol, and the treatment did not appreciably affect the body weight gains of mothers during pregnancy. Neonatal malformations were not detected. Among 2,4-dinitrophenol treated rats, however, 25 percent of the total number of young were stillborn while only 6.8 percent of the young were stillborn in the control group. In addition, the mortality during the nursing period of viable young born to mothers administering 2,4-DNP was 30.9 percent as compared with 13.4 percent for young of control mothers. Two possible explanations for this latter phenomenon were offered: treated mothers neglected their young while in a febrile state, and only the more vigorous of the offspring managed to reach the mother for nursing; or, a toxic agent was passed to the young through the milk. Data to distinguish between the two possibilities are not available.

Intraperitoneal (7.7 or 13.6 mg/kg) or oral (25.5 or 38.3 mg/kg) administration of 2,4-DNP to mice during early organogenesis does not produce

morphological defects in the young, but embryo toxicity occurs at the higher dose levels (Gibson, 1973). The higher doses also produced overt toxic signs (hyperexcitability and hyperthermia) in the dams, but were not lethal.

Bowman (1967) has studied the effect of 2,4-DNP on the developing chick embryo in vitro. At 2,4-DNP concentrations of 18 mg/l or 370 mg/l a syndrome of abnormalities resulted consisting of degeneration and sometimes complete absence of neural tissue accompanied by a reduction in the number of somites. The 2,4-DNP concentrations used in this study are extremely high and the relevance of the experimental findings to the in vivo situation in mammals is unknown.

Malformations such as hemiophthalmus and cross beak were induced in chick embryos following administration of 0.5 μ M/egg (92 μ g/egg) into the yolk sack at 48 hours of incubation (Miyamoto, et al. 1975). Based on examination of purified myelin in the malformed embryos the investigators suggested that 2,4-DNP administration resulted in deficient embryonic myelination.

Based on the available data it appears unlikely that the dinitrophenols pose a teratogenic hazard to humans. Further investigations on this question are warranted.

Mutagenicity

Friedman and Staub (1976) have developed an approach to mutagenic testing which utilizes the measurement of induction of unscheduled DNA synthesis in testes. These investigators found a good correlation between a reduction in the residual level of cell cycle-associated DNA synthesis and the presence of known mutagenic compounds. Testicular DNA synthesis in mice was unaffected by administration of 2,4-DNP suggesting a lack of mutagenic activity.

Bacterial mutagenesis of 2,4-DNP has been tested by Demerec, et al. (1951), based on the production of back mutations from streptomycin dependence to independence in E. coli. Mutations were increased severalfold over control values.

A recent study has been conducted on the effect of various phenolic compounds including 2,4-DNP on chromosomes of bone marrow cells from mice (Mitra and Manna, 1971). Mice were injected intraperitoneally with saturated aqueous solutions of 2,4-DNP and the bone marrow tissue was collected 24 hours after treatment. The results suggest that 2,4-DNP may produce chromatid type breaks in bone marrow cells. However, there was no linear relationship between the frequency of chromosome aberrations and the dose of 2,4-DNP.

It is possible to make a rough estimate of the 2,4-DNP doses administered to the mice by these investigators. The water solubility of 2,4-DNP at 75.8°F is 3.01 mg/ml (Windholz, 1976). If this value approximates the saturated solution used by Mitra and Manna (1971) and a three-to-four-month-old mouse weighs approximately 40 g, the following calculations result in three 2,4-DNP dose levels expressed as mg/kg body weight.

$$\frac{(0.25 \text{ ml}) (3.01 \text{ mg/ml})}{0.04 \text{ kg}} = 18.8 \text{ mg/kg}$$

$$\frac{(0.5 \text{ ml}) (3.01 \text{ mg/ml})}{0.04 \text{ kg}} = 37.6 \text{ mg/kg}$$

$$\frac{(1.0 \text{ ml}) (3.01 \text{ mg/ml})}{0.04 \text{ kg}} = 75.3 \text{ mg/kg}$$

The ability of 2,4-DNP to induce chromosomal damage using an in vitro alkaline elution assay employing Chinese hamster V79 cells (with or without a liver microsomal activation DNP system) was examined by Swenberg, et al. (1976). 2,4-DNP failed to induce DNA damage in this system.

Data addressing the possible mutagenicity of the other dinitrophenol isomers were not found.

Carcinogenicity

In a study designed to measure tumor promoting activity, Boutwell and Bosch, (1959) examined the ability of 2,4-DNP to promote tumor formation following a single initiating dose of dimethylbenzanthracene. Although phenol itself has a promoting activity in this system, 2,4-DNP failed to promote skin tumors in mice under similar conditions. In a similar experiment, Stenback and Garcia (1975) examined the ability of 2,4-DNP to promote skin tumor formation in mice. No promoting activity was demonstrated.

Spencer, et al. (1948) failed to detect tumor formation during chronic administration of 2,4-DNP to mice (over a six month period).

The available data suggest the 2,4-DNP does not possess carcinogenic properties. Information on the other isomeric dinitrophenols is not available.

TRINITROPHENOLS

Mammalian Toxicology and Human Health Effects

INTRODUCTION

Six isomeric forms of trinitrophenol exist distinguished by the position of the nitro groups relative to the hydroxy group on the six carbon benzene ring. The five isomers are: 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-, and 3,4,5-trinitrophenols. Production volumes for the trinitrophenols are not available. Usage of the trinitrophenol isomers is apparently limited to 2,4,6-trinitrophenol, other wise known as picric acid. In fact, a comprehensive search of the literature failed to detect a single citation dealing with any of the trinitrophenol isomers except picric acid. Consequently, the only information on these isomers presented in this document are the chemical and physical properties found in Table 8 and Figure 3.

According to Matasuguma (1967) picric acid has found use as a dye intermediate, explosive, analytical reagent, germicide, fungicide, staining agent, and tissue fixative, tanning agent, photochemical, pharmaceutical, and a process material for the oxidation and etching of iron, steal, and copper surfaces. The extent to which picric acid finds usage in any of these applications at the present time is unknown.

EXPOSURE

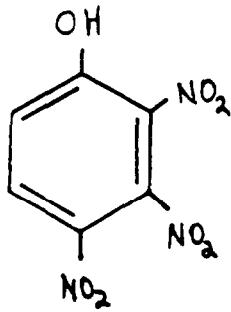
Ingestion from Water

Monitoring data on the presence or absence of 2,4,6-trinitrophenol (2,4,6-TNP) in water were not found, however, a single report of 2,4,6-TNP contamination of ground water was found (Cole, 1974). In 1955, 2,4,6-TNP (0.7 mg/l) was detected in a well approximately one mile from the former site of an expolsives manufacturing plant in England. The plant was engaged in the manufacture of explosives from 1914 to 1918. The brief report by

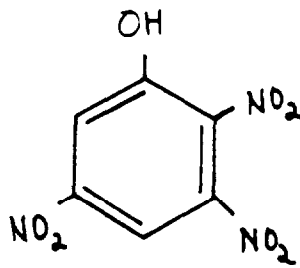
TABLE 8
Properties of Trinitrophenols*

<u>2,3,4-Trinitrophenol</u>	
Molecular Weight	229.11
<u>2,3,5-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	119-120°C
<u>2,3,6-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	119°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Very Soluble
<u>2,4,5-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	96°C
Water Solubility	
Room Temperature	Slightly Soluble
Hot Water	Soluble
<u>2,4,6-Trinitrophenol</u>	
Molecular Weight	229.11
Melting Point	122-123°C
Boiling Point	Sublimes: Explodes at 300°C
Vapor Pressure	1 mm Hg at 195°C
Density	1.763 g/cm ³
Water Solubility	
Room Temperature	1.28 g/l
100°C	6.7 g/l

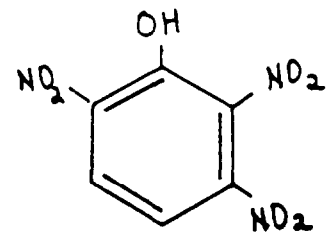
*Source: Windholz, 1976; Weast, 1975; Matsuguma, 1967.



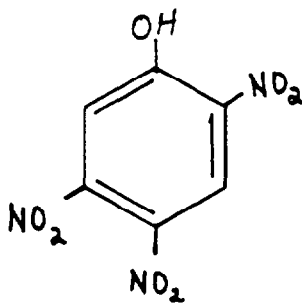
2,3,4-trinitrophenol



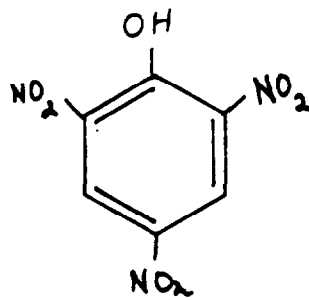
2,3,5-trinitrophenol



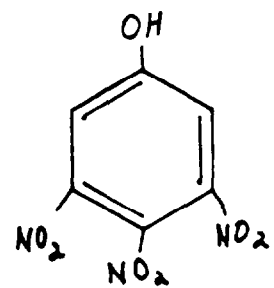
2,3,6-trinitrophenol



2,4,5-trinitrophenol



2,4,6-trinitrophenol



3,4,5-trinitrophenol

FIGURE 3
Trinitrophenols

Cole (1974) failed to describe either the types of explosive manufactured by the plant or the disposition of the waste water during the period the explosives were manufactured.

Harris, et al. (1946) described an outbreak of hematuria which resulted from ingestion of 2,4,6-TNP in the drinking water of U.S. Navy personnel aboard ships anchored at Wakayama, Japan. Approximately three weeks prior to the outbreak, more than 100 tons of confiscated Japanese ammunition, (including 2,4,6-TNP) had been dumped in the immediate vicinity of the anchorage. 2,4,6-TNP was apparently pumped into the ships' drinking water stills and carried over with the vapor phase into the freshwater supply, inducing hematuria among those who drank the water. The investigators failed to detect 2,4,6-TNP in the sea water; however, analysis of the distilled drinking water yielded 2,4,6-TNP levels to 2 to 20 mg/l.

Although it is not possible to precisely estimate either the TNP water levels or duration of exposure necessary to induce hematuria, Harris, et al. (1946) detected levels of 10 mg/l and 20 mg/l in drinking water aboard two ships at the time of the hematuria outbreak.

Hoffsommer and Resen (1973) have shown that the highly explosive tetryl (N-methyl-N,2,4,6-tetranitroaniline) dissolved in sea water at pH 8.1 and at temperature 25°C is largely converted to 2,4,6-TNP in a few months. Although tetryl is no longer manufactured in the U.S. (Howard, et al. 1976), these experiments indicate that 2,4,6-TNP may be produced in water as a result of degradation of other organic compounds. The nature of other compounds which may give rise to 2,4,6-TNP following degradation is speculative.

The persistence of 2,4,6-TNP following release to the environment is now well understood. Pitter (1976) failed to detect degradation of 2,4,6-TNP using an acclimated activated sludge system with 2,4,6-TNP as a sole source

of carbon for the microbes in the inoculum. Tabak, et al. (1964) on the other hand were able to demonstrate 95 percent degradation of 2,4,6-TNP (250 mg/l) in three to six days by acclimated cultures of microorganisms derived from garden soils, compost, and river mud. The extent to which microbial populations capable of degrading 2,4,6-TNP exist in the environment is unknown.

No other data on possible ingestion of 2,4,6-TNP from water by humans were found.

Ingestion from Food

Pertinent data could not be located in the available literature concerning exposure to 2,4,6-TNP via ingestion of food.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenols, but the equation " $\text{Log BCF} = (0.85 \text{ Log } P) - 0.70$ " can be used (Veith, et al. 1979) to estimate the steady-state BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). The log P values were obtained from Hansch and Leo (1979) or were calculated by the method described therein. The adjustment factor of $3.0/7.6 = 0.395$ is used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish in order to obtain the weighted average bioconcentration factor for the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans.

Inhalation

Pertinent information could not be located in the available literature on the presence or absence of trinitrophenols in air.

Dermal

Information on the dermal absorption of 2,4,6-TNP is scant in the literature. During the 1920s and 1930s, 2,4,6-TNP was used both alone and in

combination with butesin (di-n-butyl-p-aminobenzoate trinitrophenol) as an antiseptic surgical dressing for the treatment of burns. Ehrenfried (1911) remarked on the dangers of poisoning by absorption of 2,4,6-TNP in dermal ointments, but added that, if the ointments were properly used, there was no danger of toxic symptoms developing in humans.

A serious case of central nervous system dysfunction following the topical application of 2,4,6-TNP was reported by Dennie, et al. (1929). The patient recovered rapidly following cessation of the 2,4,6-TNP treatment. No other information on dermal absorption of the trinitrophenols by humans or experimental animals was found.

PHARMACOKINETICS

Absorption

Quantitative information on the absorption of 2,4,6-TNP by humans or experimental animals is not available.

Neurological complications following the topical administration of 2,4,6-TNP (Dennie, et al. 1929) indicate that the compound may be absorbed through the skin. Since the compound was applied to a burned area of the patient, the relevance of this data to the absorption of 2,4,6-TNP through intact skin is unknown.

The occurrence of human cases of microscopic hematuria resulting from ingestion of 2,4,6-TNP in drinking water (Harris, et al. 1946) and the known oral toxicity of 2,4,6-TNP in experimental animals indicate that absorption by the gastrointestinal tract readily occurs.

Distribution

Examination of dogs after a lethal dose of 2,4,6-TNP (Dennie, et al. 1929) revealed yellow staining of the subcutaneous fat, lungs, intestines,

and the blood vessels, indicating that 2,4,6-TNP is distributed to many tissues in the body. These investigators also demonstrated the presence of 2,4,6-TNP in the blood and suggested that the compound may be bound to serum proteins. It seems likely that distribution of 2,4,6-TNP would occur via the blood. No other data on the tissue distribution of 2,4,6-TNP following absorption were found.

Metabolism

In a review of the early literature, Burrows and Dacre (1975) indicated that elimination of 2,4,6-TNP from humans occurs in both the free form and as picramic acid. In perfusion experiments with liver, kidney and spleen, the liver exhibited the strongest capacity for reduction of 2,4,6-TNP.

Other studies dealing with the metabolism of 2,4,6-TNP in humans or in experimental animals were not found.

Decomposition of 2,4,6-TNP by an atypical strain of Corynebacterium simplex with the production of nitrites has been reported by Gunderson and Jensen (1956). This alternative metabolic pathway for 2,4,6-TNP has not been reported in mammals.

Excretion

The presence of 2,4,6-TNP in blood and urine within 1.5 hours after administration of a lethal dose in dogs was reported by Dennie, et al. (1929). The presence of 2,4,6-TNP in the urine of humans following oral exposure was reported by Harris, et al. (1946). These studies indicate that 2,4,6-TNP is partially excreted in the urine following exposure. Other data on the excretion of 2,4,6-TNP were not found.

EFFECTS

Acute, Subacute, and Chronic Toxicity

According to Windholz (1976) ingestion or percutaneous absorption of 2,4,6-TNP may cause nausea, vomiting, diarrhea, abdominal pain, oliguria,

anurea, yellow staining of skin, pruritus, skin eruptions, stupor, convulsions, and death.

Although Dennie, et al. (1929) stated: "The application of a solution of trinitrophenol to burned or abraded skin is dangerous even for nonsensitive persons since many deaths have been reported from its application," no reports of human fatalities resulting from 2,4,6-TNP exposure were found in the literature. Gleason, et al. (1968) reported the lowest recorded lethal dose for 2,4,6-TNP in humans as 5 mg/kg body weight, however, details of the poisoning episode were not provided. It is reasonable to assume, based on the known toxicity of 2,4,6-TNP in experimental animals, that exposure to sufficient amounts of the compound would be lethal in humans. The limited acute toxicity information for experimental animals has been compiled and is presented in Table 9.

Following acutely lethal doses of 2,4,6-TNP, dogs die from respiratory paralysis (Dennie, et al. 1929). Autopsy results demonstrate the presence of yellow staining of the subcutaneous fat, the lungs, the intestines, and the blood vessels. Swelling of the liver and glomerulitis of the kidneys were also seen.

The major effect of nonlethal doses of trinitrophenol (TNP) appears to be an allergic or irritative dermatitis (Anon. 1937; Ehrenfried, 1911). According to Dennie, et al. (1929) about four percent of people treated with TNP are sensitive and develop a local dermatitis. Reactions may also appear in unexposed areas. An intense itching and burning, pruritis, skin eruptions, and irritability are common. Skin eruptions are characterized by irregular-shaped macules, papules, vesicles, blebs, excoriations, and edema, as well as dried yellow crusts which are sources of reabsorption. In the maculopapular stage, a purplish-yellow color is characteristic.

TABLE 9
Acute Toxicity of Trinitrophenol Isomers^a

Species	Dose (mg/kg)	Route of Administration	Effects	References
<u>2,4,6-Trinitrophenols</u>				
Dog	100-125	s.c.	Lethal Dose	Dennie, et al. 1929
Dog	60	s.c.	MLD	Spector, 1956
Dog	60	UNK	MLD	von Oettingen, 1949
Rabbit	120	Oral	Lethal Dose	von Oettingen, 1949
Frog	200	s.c.	Lethal Dose	Windholz, 1976
Frog	200-300	s.c.	MLD	Spector, 1956
Cat	500 ^b	Oral	Lethal Dose	von Oettingen, 1949
Human	5	Oral	Lethal Dose	Gleason, et al. 1968

^aAcute toxicity data for trinitrophenol isomers other than 2,4,6-TNP were not found.

^bTotal dose in milligrams.

MLD = Minimum Lethal Dose

UNK = Unknown

More severe reactions can lead to diffuse, often severe erythema and desquamation of affected areas (Sulzburger and Wise, 1933; Am. Conf. Gov. Ind. Hyg., (ACGIH) 1971). The reaction may last from several weeks to almost a year (Sulzburger and Wise, 1933).

Effects on the skin are apparent at concentrations well below those necessary for oral systemic poisoning. Of 71 individuals exposed at concentrations of 0.0088 to 0.1947 mg/m³, dermatitis developed only among those exposed to the lower concentrations. Desensitization or adaptation reactions may occur (ACGIH, 1971).

Guinea pigs tested for allergic reactions gave similar results (Landsteiner and DiSomma, 1940; Maguire and Chase, 1972; Maguire, 1973; Chase and Maguire, 1972). Using a split-adjuvant method of sensitization, reactions have been noted at concentrations of less than one percent, with weaker solutions often giving stronger reactions. A boosting effect was also noted on subsequent tests with sensitized animals (Maguire and Chase, 1972).

Sub-lethal doses of less than or equal to 50 mg/kg body weight in dogs have resulted in transitory changes in the kidney which include glomerulitis and involvement of TNP ranging from cloudy swelling to gelatinous degeneration. The liver also showed cloudy swelling with no staining while the lungs were stained brownish-yellow in some animals (Dennie, et al. 1929).

Other reactions in humans include central nervous system effects resulting in temporary impairment of speech, memory, walking, and reflexes (Dennie, et al. 1929) and microscopic hematuria caused by ingestion of TNP (2 to 20 mg/l) in water distilled from sea water (Harris, et al. 1946).

Although it is not possible to estimate precisely either the TNP water levels or duration of exposure necessary to induce hematuria, Harris, et al. (1946) detected levels of 10 mg/l and 20 mg/l in drinking water aboard two ships at the time of the hematuria outbreak.

Crenation of intact erythrocytes by 1 mM 2,4,6-TNP has been noted by Sheetz and Singer (1976) with lysing occurring at higher concentrations. 2,4,6-TNP has also been observed to affect glycolysis in human red blood cells in vitro by affecting ATP production and consumption, although the exact mechanism has not been determined (Vestergaard-Bogind and Lunn, 1977). Pugh and Stone (1968) noted that from 6 to 11 mg/kg 2,4,6-TNP administered intravenously to anesthetized dogs results in a moderate increase in bile flow and a rise in body temperature. The clinical significance of these effects is unknown.

Synergism and/or Antagonism

Information on synergistic or antagonistic effects involving 2,4,6-TNP is scant in the literature. An interesting study by Huidobro (1971) demonstrated that administration of 50 mg of 2,4,6-TNP/kg of body weight 30 minutes before the administration of a number of analgesic drugs resulted in a significant increase in the area of analgesia induced by the opioids and the minor analgesics employed. 2,4,6-TNP did not, itself, evoke analgesia. The compounds tested included: morphine, meperidine, methadone, pentazocine, aminopyrine, sodium salicylate, and etonitazene. The investigators suggested that the elimination or metabolism of the analgesics may be modified by an effect of 2,4,6-TNP on enzymatic systems. However, further investigation is needed to definitively answer this question.

Teratogenicity

Pertinent information could not be located in the available literature on possible teratogenic effects of 2,4,6-TNP.

Mutagenicity

Streptomycin-independent mutants were induced in streptomycin-requiring E. coli B/Sd-4 after preincubation in the presence of 0.18 percent 2,4,6-TNP

for three hours before plating on streptomycin free agar (Demerec, et al. 1951) Yoshikawa, et al. (1976) reported that 2,4,6-TNP was capable of inducing mutations in Salmonella, when tested in a system which included microsomal activation. In contrast, Auerbach and Robson (1947) failed to demonstrate sex-linked lethals in Drosophila after bathing the eggs in an aqueous solution of 2,4,6-TNP. Other data on possible mutagenic properties of 2,4,6-TNP were not found.

Carcinogenicity

Pertinent data could not be located in the available literature on possible carcinogenic effects of 2,4,6-TNP.

DINITROCRESOLS

Mammalian Toxicology and Human Health Effects

INTRODUCTION

Dinitro-ortho-cresol is a yellow crystalline solid derived from o-cresol. There are six possible isomers but the 4,6-dinitro-o-cresol isomer is the only one of any commercial importance. In fact, a comprehensive search of the literature failed to reveal information on any of the other five dinitrocresol isomers.

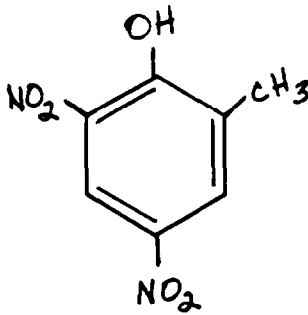
4,6-Dinitro-o-cresol (hereafter referred to as DNOC) is produced either by sulfonation of o-cresol followed by treatment with nitric acid or by treatment of o-cresol in glacial acetic acid with nitric acid at low temperature. Some important chemical and physical properties of DNOC are shown in Table 10 and Figure 4.

An excellent review of the toxicological effects of DNOC on human and laboratory animals has recently been published by the National Institute for Occupational Safety and Health (NIOSH, 1978). In view of the comprehensive coverage of both English and foreign language literature, no attempt will be made to duplicate this impressive effort within this criterion document. Key papers and frequent reference to the NIOSH review will be used where the available literature does not contain information directly relevant to criteria formulation.

DNOC usage in the U.S. has declined in recent years because the compound is highly toxic to plants in the growth stage and nonselectively kills both desirable and undesirable vegetation. Additionally, the compound is highly toxic to humans and is considered one of the more dangerous agricultural pesticides.

TABLE 10
Properties of 4,6-Dinitro-o-cresol

Molecular Weight	198.13
Appearance	Yellow Solid
Melting Point	85.8°C
Vapor Pressure	0.000052 mm Hg at 20°C
Water Solubility	100 mg/l at 20°C
pKa	4.46



4,6-Dinitro-o-cresol (DNOC)

FIGURE 4
Dinitrocresols

The Environmental Protection Agency has no record of DNOC being currently manufactured in the United States for use as an agricultural chemical. Imports of DNOC have also decreased in recent years; from 217,899 lbs. in 1972 to 146,621 lbs. in 1973 and then to 30,442 lbs. in 1976 (NIOSH, 1978). Since DNOC is not manufactured in the U.S., pesticide formulators and sprayers are the major groups with potential occupational exposure to DNOC.

DNOC is used primarily as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. NIOSH (1978) estimates that 3,000 workers in the U.S. are potentially exposed to DNOC. In view of the small amount of DNOC used in the U.S., exposure of the general public is expected to be minimal.

EXPOSURE

Ingestion from Water

Monitoring data on the presence of DNOC in ambient water are not available. An unspecified amount of DNOC was detected in the waste waters of Fison's Pest Control Limited in Harston, Cambridge, England (Jenkins and Hawkes, 1961). Webb, et al. (1973) detected 18 mg DNOC/l in the waste water of a specialty chemical plant. The extent to which human exposure to DNOC results from the ingestion of contaminated water is unknown.

Ingestion from Food

No data are available on the presence or absence of DNOC residues in food for human consumption. Since the primary usage of the compound involves treatment of fruit trees during the dormant season, it appears unlikely that contamination of human food stuffs would occur to any large extent.

No measured steady-state bioconcentration factor (BCF) is available for any nitrophenols, but the equation " $\text{Log BCF} = (0.85 \text{ Log } P) - 0.70$ " can be

used (Veith, et al. 1979) to estimate the steady-state BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). The log P values were obtained from Hansch and Leo (1979) or were calculated by the method described therein. The adjustment factor of $3.0/7.6 = 0.395$ is used to adjust the estimated BCF from the 7.5 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish in order to obtain the weighted average bioconcentration factor for the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans.

Inhalation

An evaluation of the literature (NIOSH, 1978) indicates that occupational injury and disease associated with exposure to DNOC results primarily from inhalation of, and skin contact with, the aerosol form. A large number of human intoxications, including fatalities, have been reported resulting from such DNOC exposure. Persons at risk include those manufacturing, formulating, or applying the compound as an aerosol. Inhalation exposure to the general public is expected to be minimal although data addressing this point are not available.

Dermal

As mentioned in the preceding section, occupational intoxication by exposure to DNOC has occurred as a result of inhalation and dermal exposure where the compound is manufactured, formulated or applied. Dermal exposure of the general public is considered unlikely, however, direct data bearing on this point were not found.

PHARMACOKINETICS

Absorption

DNOC is readily and rapidly absorbed through the skin, the gastrointestinal tract and respiratory tract in humans (NIOSH, 1978). Although most

cases of occupational intoxication resulting from DNOC exposure contain both a respiratory and a dermal component, human intoxication has been reported as a result of dermal contact with DNOC alone.

In a report from the Russian literature (Buchinskii, 1974) a four-year-old boy was fatally intoxicated after a rash had been treated with 50 g of an ointment to which 25 percent DNOC was added by mistake. Stott (1956) reported two cases of DNOC poisoning resulting from skin absorption. The two men were involved in the cleaning and maintenance of aircraft booms used to spray solutions of DNOC. Since neither man worked near the actual operation, and both denied blowing into the spray jet to clean them, Scott (1956) concluded that the major route of exposure was skin contact.

Work by Harvey, et al. (1951) indicates that DNOC is rapidly absorbed by the human gastrointestinal tract. These investigators described the effects of DNOC taken orally by five male volunteers. It was noted the DNOC levels in the blood increased steadily after administration and were maximal from two to four hours after ingestion. Van Noort, et al. (1960) investigated the effectiveness of personal protective equipment used by 24 sprayers in Holland. Serum DNOC levels and the quantity of DNOC used were determined in a three-week spraying period. Their findings indicated that both inhalation and dermal contact with DNOC can lead to an appreciable absorption into the blood stream.

Experimental animal studies, reviewed by NIOSH (1978), also have confirmed the toxicity of DNOC in humans exposed by the oral, inhalation, and dermal routes.

Distribution

Whether absorption of DNOC occurs through the skin, gastrointestinal tract, or respiratory tract, the compound is transported in and distributed by the blood (NIOSH, 1978). Harvey, et al. (1951) described the effect of

DNOC taken orally by five male volunteers. Capsules containing 75 mg of pure DNOC were administered daily for five consecutive days amounting to a total dose of from 0.95 to 1.27 mg/kg/day. The concentration of DNOC in the blood increased in the first three to four days and reached concentrations of 15 to 20 mg/kg. After concentrations of 15 to 20 mg/kg had been obtained, additional doses appeared to cause temporary high blood concentrations which were associated with toxic symptoms.

Blood analysis of humans displaying symptoms of DNOC toxicity has invariably revealed concentrations exceeding 10 mg/kg (NIOSH, 1978).

In studies conducted to determine the kinetics of absorption and distribution, DNOC has not been shown to accumulate in the blood of various animal species (King and Harvey, 1953a; Parker, et al. 1951). In rats and rabbits that were given two or more daily injections of DNOC subcutaneously, serum levels on succeeding days were no higher than they were 24 hours after the first dose (Parker, et al. 1951). Serum levels in dogs rose for the first three days but then decreased despite the administration of two additional doses.

DNOC is more rapidly eliminated from the blood of animals than from the blood of humans (King and Harvey, 1953b; Parker, et al. 1951; Harvey, et al. 1951). Within a 24-hour period following a single subcutaneous injection of DNOC, elimination from the serum of rabbits was nearly complete. Four days were necessary for serum clearance in rats and cats, while six days were required for elimination from the serum of dogs (Parker, et al. 1951). DNOC accumulated only slightly in the blood when given to rats by stomach tube or i.p. injection and did not accumulate in the blood of rabbits after administration by stomach tube (King and Harvey, 1953a).

The accumulation of DNOC in the blood of humans following DNOC exposure has been well documented (Harvey, et al. 1951; Bidstrup, et al. 1952). The accumulative effect may reflect the binding of DNOC with albumin in the blood and a subsequent slow rate of excretion in humans (Harvey, et al. 1951).

DNOC is slowly eliminated from humans. The investigations by Harvey, et al. (1951) indicated detectable amounts of DNOC in the blood (1 mg/kg) as long as 40 days following the last of five consecutive daily oral doses in human volunteers. Another study (Van Noort, et al. 1960; reviewed by NIOSH, 1978) showed that it took two to eight weeks for DNOC to be cleared from the serum.

Parker, et al. (1951) studied the tissue distribution of DNOC following subcutaneous injection in the rat. They noted that a single dose of 10 mg/kg DNOC produced very high levels in the serum (100 mg/l at 30 min) but no accumulation in other tissues was detected. The lungs and heart contained high levels of DNOC but the investigators postulated that these levels were the highest due to the high blood content of these organs. The investigators calculated that within 30 minutes of the injection, 83 percent of the DNOC that could be accounted for was present in the blood. Six hours after the injection, 0.37 mg of the 1.5 mg dose of DNOC could be accounted for, of which 72 percent was in the blood.

DNOC content of a number of tissues was determined in rats receiving a single subcutaneous injection of the compound (Parker, et al. 1951). The results, presented in Table 11, clearly indicate the DNOC failed to accumulate in the tissues.

In another experiment Parker, et al. (1951) failed to detect significant DNOC accumulation in liver or kidney tissue of rats after 40 successive daily injections of 20 mg/kg DNOC.

TABLE 11*

DNOC Content of Blood and Tissues** of Rats Killed at Intervals After
Subcutaneous Injection of One Dose of 1.5 mg DNOC*

Time After Injection	Serum (mg/l)	Brain	Spleen	Kidney	Liver	Muscle	Heart	Lung
30 min.	100	1.5	4.0	7.5	14.0	0.5	98.0	18.0
1 hr.	89	3.5	4.0	7.5	12.0	2.0	13.5	20.0
2 hrs.	97	2.0	4.5	11.0	10.5	0.0	19.0	20.5
3 hrs.	93	4.0	8.0	11.0	11.5	3.5	14.0	15.5
4 hrs.	79	3.5	3.0	4.5	13.5	0.5	13.0	14.0
5 hrs.	76	2.0	4.0	4.5	8.5	2.0	14.0	14.5
6 hrs.	45	3.0	1.5	7.5	8.5	1.5	10.5	30.0

*Source: Parker, et al. 1951.

**DNOC content of tissue mg/kg net weight.

In a single study reviewed by NIOSH (1978) Sovljanski, et al. (1971) discussed tissue distribution of DNOC in humans. Autopsy results of two victims, who had committed suicide by ingestion of DNOC, yielded detectable DNOC in the stomach, intestines, liver, kidneys, heart, and brain, with the stomach containing the greatest amount. Neither blood DNOC levels nor quantitative data on tissue levels were reported.

Steer (1951), on the other hand, demonstrated that the tissues of a fatal case of DNOC poisoning contained no more than 5 mg/kg of DNOC and many contained 1 mg/kg or less.

According to King and Harvey (1953b) the accumulation of DNOC in man can be explained in two ways; either the detoxification and excretion are very slow or there is some storage of DNOC in body tissues. Based on their calculation of excretion kinetics in man, the investigators suggested that detoxification and excretion of DNOC are inefficient and slow in humans.

None of the available data suggest significant accumulation of DNOC in specific tissues of humans or experimental animals (NIOSH, 1978).

Metabolism

The metabolism of DNOC in humans has not been studied. However, several investigators have conducted experiments to determine the rate of DNOC after its administration to animals.

Truhaut and De Lavour (1967) reported on the metabolism of DNOC in rabbits. Following the administration of DNOC by gastric intubation, both DNOC and 6-amino-4-nitro-o-cresol were detected in liver, kidney, brain, and urine of animals. 4-Amino-6-nitro-o-cresol was not detected in the animals. It was concluded by the investigators that the ratio of 6-amino-4-nitro-o-cresol to DNOC in the tissue and urine was a function of the dose of DNOC administered to the animal. When a low dose of DNOC was administered,

very little 6-amino-4-nitro-o-cresol was detected in either the urine or tissues. The authors considered the metabolism of DNOC to 6-amino-4-nitro-o-cresol a detoxification mechanism that plays an important role only when a toxic dose of DNOC is administered. They further suggested that the ratio of 6-amino-4-nitro-o-cresol to DNOC might be a useful indicator in evaluation of the severity of exposure to DNOC.

The metabolic fate of DNOC in rabbits was also investigated by Smith, et al. (1953). Following administration of 20 to 30 mg/kg DNOC to rabbits by stomach tube, urinary metabolites were identified by paper chromatography and spectrophotometry. Less than 20 percent of the dose was recovered in the urine in two days. Between 5 and 5.5 percent was detected as free DNOC, and 0.7 percent as DNOC conjugates. The conjugates were not characterized by the investigators. Most of the urinary metabolites (about 12 percent of the dose) were derivatives of 6-amino-4-nitro-o-cresol. About 1.5 percent of the dose was excreted as 6-acetamido-4-nitro-o-cresol, and 9 to 10.5 percent as the hydroxyl group conjugate. Traces of 6-amino-4-nitro-o-cresol, 4-amino-6-nitro-o-cresol, and 3-amino-5-nitrosalicylic acid were also detected.

Since the detoxification and excretion of DNOC in man are very slow compared to rats or rabbits (King and Harvey, 1953b), the applicability of the experimental animal detoxification mechanism to the human situation is unknown. The elucidation of DNOC detoxification mechanism in humans awaits further investigation.

Excretion

Available data indicate that DNOC is rapidly excreted following administration to experimental animals. Parker, et al. (1951) found that DNOC injected subcutaneously disappeared from the blood at various rates in differ-

ent species. Single 10 mg/kg doses of DNOC were administered subcutaneously to an unspecified number of dogs, cats, rabbits, and rats. DNOC given in one injection was completely eliminated from the serum of rabbits within 24 hours, while blood DNOC levels were between 30 and 40 mg/l in the rats, cats, and dogs at this time. It took four days for DNOC blood levels to fall to zero in rats and cats, and six days in dogs. The half-time for elimination of DNOC from the blood after a single injection of 10 mg/kg DNOC was approximately three hours in the rabbit, 15 hours in the rat, 20 hours in the cat, and 36 hours in the dog.

Lawford, et al. (1954) reported that animals eliminated DNOC from the blood in the following descending order of efficiency: mouse, rabbit, guinea pig, rat, and monkey.

DNOC is eliminated in the blood of animals faster than it is from the blood of humans (King and Harvey, 1953b; Parker, et al. 1951). King and Harvey (1953b) calculated the half-time for elimination of DNOC from the blood of rats, rabbits, and humans. The values were 28.5 hours, 6.6 hours, and 153.6 hours, respectively.

Pollard and Filbee (1951) reported on the urinary excretion of DNOC from a seriously poisoned man in Great Britain. The man was admitted to the hospital and full biochemical investigations were performed immediately after admission. The man recovered almost totally from the poisoning episode within five days. However, DNOC levels of 4 mg/l were still detected in the blood one month following the exposure. Blood DNOC level was reported to fall in an exponential fashion.

Van Noort, et al. (1960) measured the serum DNOC levels in ten spraymen on a weekly basis for two months after the spraying period ended. They found the DNOC was eliminated from the serum slowly and that the rate varied

from individual to individual. Two to eight weeks elapsed before DNOC was cleared completely from the serum of these workers. The amount of time needed for DNOC to be totally eliminated was directly related to the quantity of DNOC in the serum on the last day of exposure.

In experiments where DNOC was orally administered to five human volunteers, Harvey, et al. (1951) demonstrated that DNOC, absorbed by ingestion at 24-hour intervals accumulates in the human body and is excreted slowly. Forty days after the last dose of DNOC was orally administered by mouth, 1 to 1.5 mg/l DNOC was still present in the blood.

The experimental evidence suggests, therefore, that a substantial difference in the excretion patterns of humans vs. experimental animals exists. Since storage of DNOC in the tissues of humans has not been reported, it is concluded that slow and inefficient detoxification or excretion probably occurs in humans.

Occupational studies (NIOSH, 1978) have long utilized serum levels of DNOC in order to assess exposure of humans to dangerous amounts of the compounds. A review of the literature (NIOSH, 1978) indicates that workers with DNOC concentrations of 40 mg/kg of whole blood (approximately 80 mg/l of serum) or greater will most likely develop toxic effects. In addition, in the concentration range between 20 and 40 mg/kg of whole blood (probably because of variation in individual susceptibility) some workers are affected and others show no adverse effects. Most workers with blood DNOC levels below 20 mg/kg are not affected, although because of individual susceptibility, some exhibited mild effects. The blood level of 20 mg/kg has been used as a maximum permissible level for industrial or agricultural workers utilizing the compound during employment.

Bidstrup, et al. (1952) recommended that a person should be removed from further contact with DNOC for at least six weeks if the blood level eight hours after the last exposure was 20 mg/kg or higher.

Other data on the elimination of DNOC from humans were not found.

EFFECTS

Acute, Subacute, and Chronic Toxicity

The acute toxic dose of DNOC with different routes of administration, has been determined for a number of different experimental animal species. These data have been compiled and presented in Table 12.

Although the available human toxicity data do not permit the calculation of the acute lethal dose for DNOC in humans, it has been estimated (Fairchild, 1977) that 5 mg/kg may prove lethal to humans.

A large number of occupational and nonoccupational poisonings of humans by DNOC have been reviewed by NIOSH (1978). The available literature concerning humans indicates that DNOC may be absorbed in acutely toxic amounts through the respiratory and gastrointestinal tracts and through the skin, and that it accumulates in the blood. Individuals exposed to DNOC by these routes usually demonstrate signs of increased metabolic rate. Symptoms of poisoning include profuse sweating, malaise, thirst, lassitude, loss of weight, headache, a sensation of heat, and yellow staining of the skin, hair, sclera, and conjunctiva.

In addition to the effects associated with increased metabolism, other effects occasionally reported in humans poisoned by DNOC included kidney damage, diarrhea, unspecified changes in the gastrointestinal tract, in the cardiovascular system, and in the peripheral vascular and central nervous systems.

TABLE 12
Acute Toxicity of 4,6-Dinitro-o-cresol

Species	Dose (mg/kg)	Route of Administration	Effects	References
Mouse	187	Dermal	LD50	Arustamyan, 1972
Rabbit	1000	Dermal	LD50	Burkatskaya, 1965
Guinea Pig	500	Dermal	100% Lethal	Spencer, et al. 1948
Rat	85	Oral	LD50	Burkatskay, 1965
Rat	30	Oral	MLD	Ambrose, 1942
Rat	40	Oral	100% Lethal	Ambrose, 1942
Rat	30	Oral	LD50	Spencer, et al. 1948
Mouse	47	Oral	LD50	Burkatsukaya, 1965
Mouse	16.4	Oral	LD50	Arustamyan, 1972
Hare	24.8	Oral	LD50	Janda, 1970
Cat	50	Oral	LD50	Burkatsukaya, 1965
Pheasant	8.4	Oral	LD50	Janda, 1970
Partridge	8.3	Oral	LD50	Janda, 1970
Rat	26-39	s.c.	LD50	Harvey, 1952
Rat	20	s.c.	MLD	Ambrose, 1942
Mouse	24.2	s.c.	LD50	Parker, et al. 1951
Rat	24.6	s.c.	LD50	Spector, 1956
Goat	50	s.c.	LD50	Ambrose, 1942
Dog	15	i.v.	LD	Spector, 1956
Dog	5	i.m.	LD	Spector, 1956
Dog	10	i.p.	LD	Spector, 1956
Pigeon	5	i.m.	LD	Spector, 1956

MLD = Minimum Lethal Dose

s.c. = subcutaneous

i.m. = intramuscular

i.v. = intravenous

i.p. = intraperitoneal

It is generally believed that the toxic effects of DNOC result from its ability to uncouple the oxidative phosphorylation process. DNOC is an extremely potent uncoupler of oxidative phosphorylation. This effect results in the decreased formation of adenosine triphosphate (ATP) and a resulting inhibitory effect of enzyme reactions requiring ATP. Such a toxicant is expected to have extreme and profound effects on all tissues where the concentration of the chemical is high enough to severely affect oxidative phosphorylation. Since energy generated in the body cannot be converted to its usual form (ATP) in the presence of DNOC, it is released as heat instead, causing many of the commonly observed signs and symptoms of DNOC toxicity.

Several investigators have correlated blood DNOC levels with the severity of toxic effects in humans (Harvey, et al. 1951; Bidstrup, et al. 1952; Pollard and Filbee, 1951) and have shown that, unlike the situation in animals, DNOC accumulates in the blood of humans. Accumulation is believed to occur as a result of DNOC binding to albumin in the blood (Harvey, et al. 1965). In one of the few cases where DNOC in the blood of a poisoned human was monitored throughout his recovery period, (Pollard and Filbee, 1951) the severity of the symptoms decreased as blood levels of DNOC decreased. Data on blood DNOC levels in humans and the accompanying effects are compiled and presented in Table 13. The data show that workers with DNOC concentrations of 40 mg/kg of whole blood (approximately 80 mg/l of serum) or greater will most likely develop toxic effects. In the concentration range between 20 and 40 mg/kg of whole blood, some workers are affected and others show no adverse effects (probably because of differences in individual susceptibility). Most individuals with blood levels of DNOC below 20 mg/kg were not affected, although some exhibited mild effects. As the data in Table 4 suggest, most investigators have concluded that blood DNOC levels are associated with the severity of intoxication in humans (NIOSH, 1978).

TABLE 13

Relationship to Blood DNOC Levels and Effects in Humans*

Route Exposure	No. of Individuals and Occupation	Blood DNOC Level (mg/kg)	Effects
Inhalation, Dermal	1 Agricultural Worker	1000 ^{a,b}	Death
Inhalation, Dermal	1 Agricultural Worker	200 ^{a,b}	Sweating, labored breathing, vomiting
Inhalation, Dermal	1 Agricultural Worker	75	Death
Inhalation, Dermal	1 Agricultural Worker	60	Headache, lassitude, BMR 275%
Inhalation, Dermal	1 Agricultural Worker	60 ^{a,b}	Sweating, headache, labored breathing, fatigue
Inhalation, Dermal	1 Agricultural Worker	55	Unconsciousness
Inhalation, Dermal	2 Agricultural Workers	44-55	Acute Poisoning
Oral	5 Experimental Subjects	40-48	Headache, lassitude, malaise
Inhalation, Dermal	4 Agricultural Workers	20-40 ^a	Liver damage
Inhalation, Dermal	5 Agricultural Workers	30-40	No effects
Inhalation, Dermal	6 Agricultural Workers	21-40 ^a	Moderate poisoning; recovery period longer than 8 days
Inhalation, Dermal	32 Agricultural Workers	7-37 ^a	Mild poisoning; recovery within 8 days
Inhalation, Dermal	1 Agricultural Worker	30 ^a	Fever
Inhalation, Dermal	16 Agricultural Workers	20-30	No effects
Inhalation, Dermal	1 Agricultural Worker	25 ^a	Kidney damage
Inhalation, Dermal	21 Agricultural Workers	10-20	No effects
Inhalation, Dermal	149 Agricultural Workers	<10	No effects
Inhalation, Dermal	4 Agricultural Workers	4-9 ^{a,b}	Sweating, thirst
Inhalation, Dermal	23 Agricultural Workers	1-8 ^{a,b}	No effects
Inhalation, Dermal	1 Agricultural Worker	<5 ^{a,b}	Fatigue
Inhalation, Dermal	2 Manufacturing Workers	10-20	No effects
Oral	5 Experimental Subjects	20	Exaggerated feeling of well-being

*Source: NIOSH, 1978

^aReported as mg/l^bSerum or Plasma DNOC Level

BMR = Basal Metabolic Rate

In comparing studies on blood DNOC levels, certain precautions must be taken when correlating the results. It has been reported that over 90 percent of the DNOC detected in the blood is found in serum (Parker, et al. 1951) and that most of this amount is bound to albumin in humans (Harvey, et al. 1951). A comparison of numerically similar blood DNOC levels expressed as weight/volume of serum with those expressed as weight/weight of whole blood can therefore only be done by approximate conversions. Any given DNOC serum level will have a lower value when expressed per unit of whole blood.

It is impossible to develop a dose-response relationship for occupational DNOC poisoning in humans since air concentrations of DNOC are rarely reported and the exposure time of poisoned individuals is highly variable. In most cases of human poisoning total exposure amounts can only be estimated. This lack of data makes assessment of a minimum toxic dose for humans extremely difficult. Several studies however, where the oral toxicity of DNOC has been assessed in humans, shed some light on this question.

Harvey, et al. (1951) orally administered DNOC to five male volunteers and studied both the resulting blood levels and toxic effects. Each man was given capsules containing 75 mg of pure DNOC daily for five consecutive days, amounting to a total dose of 0.92 to 1.27 mg/kg/day. The men experienced an exaggerated sense of well-being when blood levels were about 20 mg/kg. Headache, lassitude, and malaise were associated with DNOC blood levels of 40 to 48 mg/kg. Although individual variation was evident in these experiments, it is obvious that chronic administration of 1 mg/kg/day DNOC to healthy humans may result in signs of toxicity. The exaggerated sense of well-being described by Harvey, et al. (1951) is a typical sign of impending toxic effects among agricultural workers exposed to DNOC.

DNOC was introduced in 1933 as an alternative to dinitrophenol for the treatment of obesity (NIOSH, 1978). Many poisonings, and some deaths, resulting from overdoses were reported, as well as the development of cataracts in some patients, months after they had stopped taking DNOC. Some patients developed symptoms of DNOC poisoning at the accepted therapeutic dose level. Signs and symptoms of DNOC intoxication including thirst, fatigue, excessive sweating, decreased appetite, and elevated basal metabolic rates, appeared in three persons who had taken as little as 0.35 to 1.5 mg/kg/day of DNOC for up to 9 weeks (Plotz, 1936). Hunter (1950) noted that, although, less than one percent of those individuals treated with DNOC developed complications, he considered the difficulty of setting a safe dose for each individual to be the reason that its use as an aid to weight loss was discontinued.

Although DNOC is considered a cumulative poison in humans, probably as a result of slow metabolism and inefficient excretion, true chronic or sub-acute effects (with the possible exception of cataract formation) have never been reported in either human or experimental animals. Signs and symptoms of toxicity occur when the total body burden exceeds a threshold level. The toxic effects noted after either acute or chronic administration are similar in quality and their severity appears to be correlated with DNOC blood levels (and by inference, total body burden). It is generally agreed that the toxic manifestations of DNOC result from its potent effects on metabolism (NIOSH, 1978).

Several long-term studies designed to determine dietary levels of DNOC necessary to cause toxic symptoms in experimental animals have been conducted. Spencer, et al. (1948) maintained rats on a diet containing DNOC for six months. Growth curves, periodic blood counts, analyses of urea-

nitrogen, organ weights, and histopathological examinations were performed on all animals. No adverse effects on these parameters were detected among rats fed diets containing 100 mg DNOC/kg food. Higher concentrations in the diet produced effects that may be attributed to the action of DNOC as a metabolic stimulant. Such effects included: weight loss or poor weight gain, marked emaciation, a hungry, thin, and unkempt appearance, and minor histopathological effects on the liver, kidneys, and spleen at the highest dose level (1,000 mg DNOC/kg food). For water, a concentration of approximately one-half the dietary intake will result in the equivalent dosage on a body weight basis (assuming a fluid intake two times the dry matter intake). Thus, the no-observable-effect-level for DNOC in rats, if all DNOC were derived from drinking water, would be 200 mg/l.

In a similar study, Ambrose (1942) reported no observable effect on rats fed diets containing 63 mg DNOC/kg food for 105 days. At DNOC levels of 125 mg/kg food, 60 percent of the animals died. At necropsy and histopathological examination, the tissues of all rats receiving the drug for 30 days or more failed to show any characteristic lesions that could be ascribed to the drug. The no-effect-level calculated for DNOC in drinking water was 126 mg/l.

When DNOC was administered in the diet of rats by Parker, et al. (1951) poisoning was only observed when the calculated daily intake of the drug greatly exceeded the single lethal dose. At a level of 200 mg DNOC/kg food, rats grew normally during an observation period of 18 weeks.

A federal workplace environmental limit of 0.2 mg/m³ for DNOC has been recommended by NIOSH (1978). The limit was based on the following considerations: a study from the Russian literature (Burkatskaya, 1965) documented the lowest airborne DNOC levels, found in the literature, associated with

health effects in humans. Exposure to airborne DNOC at concentrations that averaged 0.9 mg/m^3 produced unspecified changes in the cardiovascular system, the central and autonomic nervous systems, the gastrointestinal tract, and the cell pattern of the peripheral blood of workers involved in manufacturing and applying DNOC. In agricultural workers exposed to DNOC at an average concentration of 0.7 mg/m^3 , slight unspecified changes in the blood and autonomic nervous system were observed.

Another study (Batchelor, et al. 1956) revealed that agricultural sprayers exposed to an airborne DNOC concentration about 0.23 mg/m^3 failed to demonstrate adverse effects of the compound. No symptoms of poisoning were observed and blood DNOC levels were well below those associated with toxic effects.

In the study by Burkatskaya (1965) the effect of airborne DNOC on cats was examined. Cats exposed at 0.2 mg/m^3 for two or three months had slightly increased body temperatures and leucocyte counts and decreased hemoglobin concentrations, erythrocyte counts, and catalase and peroxidase activities. The changes, which were characterized as slight and transient, occurred after one to two weeks but further exposure produced no additional effects.

The report by NIOSH (1978) concludes "since only slight effects were seen in workers exposed to DNOC at an average concentration as low as 0.7 mg/m^3 for an unspecified duration, and since short-term exposure at 0.2 mg/m^3 had no lasting effect on cats." NIOSH recommends that the current federal workplace environmental limit of 0.2 mg/m^3 be retained.

It is possible to calculate the anticipated daily exposure of a 70 kg human male exposed to DNOC at 0.2 mg/m^3 for an 8-hour period. If one assumed the average minute volume was 28.6 l of air/minute (NIOSH, 1978) the anticipated daily exposure is $39 \text{ } \mu\text{g/kg/day}$.

If one assumes that absorption of DNOC across the respiratory tract is identical to gastrointestinal absorption, and that a 70 kg human male consumes 2.0 liters of water daily, the following calculation indicates the maximum allowable levels of DNOC in drinking water based on the NIOSH recommendation for workplace air.

$$39 \text{ ug/kg/day} \times 70 \text{ kg} = 2.73 \text{ mg/day}$$

$$\frac{2.75 \text{ mg/day}}{2 \text{ l/day}} = 1.38 \text{ mg/l}$$

Although NIOSH (1978) states "the standard was not designed for the population-at-large, and any extrapolation beyond the occupational environment is not warranted," development of a baseline level for chronic human effects using the same data used by NIOSH appears to be a reasonable approach to the development of a water criterion.

In summary, daily human exposure to 0.35 mg/kg DNOC may result in signs of intoxication in humans. Some persons develop cataracts as a result of chronic exposure to DNOC, but the no-effect-level for cataracts cannot be calculated. Although true "chronic" effects of DNOC have never been documented, the compound accumulates in the human body and toxic symptoms may develop when blood levels exceed 20 mg/kg. Such symptoms have been observed in humans receiving as little as 0.35 mg/kg/day over a period of several weeks. The no-observable-effect-level for rats in long-term feeding studies has been variously reported as 63 mg/kg food, 100 mg/kg food, and 200 mg/kg food. Based on the available human and experimental animal data, NIOSH (1978) has recommended a federal workplace limited of 0.2 DNOC/m³ air. Based on an estimate of human exposure for an eight-hour work shift, it was calculated that a drinking water level of 1.4 mg/l would result in a similar exposure to the general population.

Synergism and/or Antagonism

Pertinent information could not be located in the available literature describing synergistic or antagonistic effects associated with DNOC.

Teratogenicity

Pertinent information could not be located in the available literature regarding the presence or absence of teratogenic properties of DNOC.

Mutagenicity

Andersen, et al. (1972) reported an evaluation of the ability of 110 herbicides, including DNOC, to produce point mutations in histidine-dependent mutants of Salmonella typhimurium, bacteriophage T4, and in two RII mutants of bacteriophage T4. The culture media were prepared by mixing freshly grown cultures of the mutants with soft agar and pouring into petri dishes. After the agar solidified, DNOC was applied to the surface of each plate. They found that the mutation frequency rates produced by DNOC were no greater than the spontaneous rates.

Nagy, et al. (1975) tested DNOC for its ability to induce back-mutations of her^+ and her^- derivatives of E. coli WP2 Try- bacteria. DNOC failed to induce reverse mutations in this system.

The difference in growth inhibitions of wild type Proteus mirabilis and the corresponding repair-deficient strain has been used by Adler, et al. (1976) as an indication of DNA damage. Evidence of DNA damage in the presence of DNOC was reported.

Information on the potential mutagenicity of DNOC for mammals is not available.

Carcinogenicity

Spencer, et al. (1948) failed to report tumor formation in rats maintained on diets containing DNOC for six months. Similarly, no tumors were reported in rats maintained on diets containing DNOC for 105 days (Ambrose, 1942) or 126 days (Parker, et al. 1951).

No further information was found regarding the presence or absence of carcinogenic properties of DNOC.

CRITERIA FORMULATION

Existing Guidelines and Standards

U.S. standards for exposure to the nitrophenols or dinitrocresols in drinking or ambient water have not been set.

The following limits for toxic substances in drinking water have been set in the USSR (Stofen, 1973):

2-nitrophenol	0.06 mg/l
3-nitrophenol	0.06 mg/l
4-nitrophenol	0.02 mg/l
2,4-dinitrophenol	0.03 mg/l

Based on organoleptic considerations, a limit of 0.5 mg/l for 2,4,6-trinitrophenol has been set by the USSR (Stofen, 1973).

The maximum air concentration established by the American Conference of Governmental Industrial Hygienists (ACGIH, 1971) is 0.1 mg/m³ for 2,4,6-trinitrophenol and 0.2 mg/m³ for 4,6-dinitro-o-cresol for an eight-hour exposure (TLV).

The Code of Federal Regulations (40 CFR Part 180) establishes a tolerance of 0.02 mg/kg for residues of 4,6-dinitro-o-cresol and its sodium salt in or on apples resulting from applications to apple trees at the blossom stage as a fruit-thinning agent.

Current Levels of Exposure

Human exposure to the nitrophenols or dinitro-o-cresols has not been monitored. Unspecified amounts of 4-nitrophenol have been detected in samples of urban ambient particulate matter.

The photochemical reaction between benzene vapor and nitrogen monoxide results in the production of 2-nitrophenol, 4-nitrophenol, 2,4-nitrophenol, and 2,6-dinitrophenol under laboratory conditions and 4-nitrophenol has been

detected in rainwater in Japan. Available data indicate that the general public may be exposed to nitrophenols in the atmosphere when severe photochemical fog conditions develop. Quantitative estimates of such exposures are not possible at the present time.

4-Nitrophenol has been detected in the urine of 1.0 percent of the general population at levels as high as 0.1 mg/l (with a mean urinary level of 10 µg/l).

If it is assumed that urinary residues of 4-nitrophenol reflect direct exposure to the compound, a pharmacokinetic estimate of exposure based on steady-state conditions can be made. The exposure level leading to the 1.0 µg/l residue can be calculated as follows.

$$\text{Exposure} = \frac{(10 \text{ } \mu\text{g/l}) (1.4 \text{ l of urine/day})}{(70 \text{ kg/man})} = 0.02 \text{ } \mu\text{g/kg/day}$$

A similar calculation using the maximum urine residue level observed (113 µg/l) gives an exposure of 2.26 µg/kg/day.

However, these urine levels are not believed to result from direct exposure to 4-nitrophenol. A number of widely used pesticides, including parathion, are readily metabolized to 4-nitrophenol in the human body and are believed to be the source of 4-nitrophenol residues in human urine.

Current levels of human exposure to the nitrophenols or dinitrophenols (with the possible exception of 4-nitrophenol) are either very low, nonexistent, or have gone undetected. In the absence of data any of the above could be operative.

Special Groups at Risk

The only individuals expected to be at risk for high exposure to the nitrophenols are industrial workers involved in the manufacture of compounds for which the nitrophenols are intermediates. Since picric acid (2,4,6-tri-

chlorophenol) may find some use as an explosive, germicide, tanning agent, fungicide, tissue fixative, or industrial process material, a higher risk of exposure exists among personnel engaged in such operations.

Although 4,6-dinitro-o-cresol (DNOC) is no longer manufactured in the U.S., a limited quantity is imported and used as a blossom-thinning agent on fruit trees and as a fungicide, insecticide, and miticide on fruit trees during the dormant season. Hence, individuals formulating or spraying the compound incur the highest risk of exposure to the compound.

Basis and Derivation of Criterion

The organoleptic thresholds for mononitrophenols in water range from 0.24 to 389 mg/l. These levels, extracted from the Russian literature, are detection thresholds; acceptability thresholds from the standpoint of human consumption are not available.

With the exception of a single study abstracted from the Russian literature, data on chronic mammalian effects of the mononitrophenols are absent from the literature.

The Russian investigation (Makhinya, 1969) was reported in abstract form only. Attempts to obtain the full report proved fruitless. The investigators reported distinct cumulative toxic properties of the mononitrophenol isomers in mammals. Threshold levels for effects of mononitrophenols on conditioned reflexes were reported, but details of the experiment including animal species, mode of administration, duration of the experiment, and the exact parameters measured are not available. Hence, it does not seem prudent to develop a criterion based on these results.

In the absence of data on chronic mammalian effects no water criterion for human health can be established for any of the mononitrophenol isomers at this time.

Information on the dinitrophenol isomers is limited to 2,4-dinitrophenol. Spencer, et al. (1948), in a six-month feeding study with rats administered 5 dietary levels of 2,4-dinitrophenol, demonstrated the no-observed-effect-level (NOEL) to be between 5.4 mg/kg and 20 mg/kg. Using the lower of the two figures and assuming a 70 kg man consumes 2 liters of water daily and 6.5 grams of contaminated fish having a BCF of 1.51 the corresponding NOEL for humans based on the results obtained in rats may be calculated as follows:

$$\frac{5.4 \text{ mg/kg} \times 70 \text{ kg} = 378 \text{ mg}}{2 \text{ liters} + (1.51 \times 0.0065) \times 1.0} = 188 \text{ mg/l}$$

Based on these calculations, no biological effect would be predicted in a man drinking water containing 2,4-DNP at 188 mg/l.

Experience with the use of 2,4-DNP as an anti-obesity drug in the 1930's indicates that adverse effects, including cataract formation, may occur in humans exposed to as little as 2 mg/kg/day. The drug was frequently used in an uncontrolled manner and the available data do not allow the calculation of a no-adverse-effect-level in humans. It is clear, however, that ingestion of 2,4-DNP at 2 mg/kg/day for a protracted period may result in adverse effects, including cataracts, in a small proportion of the population. This dietary intake level constitutes a low-observed-adverse-effect-level (LOAEL). Assuming a 70 kg man consumes 2 l of water daily and 6.5 grams of contaminated fish having a BCF of 1.51 and assuming 100 percent gastrointestinal absorption of 2,4-DNP, a 2 mg/kg dose of 2,4-DNP would result if drinking water contained 2,4-DNP at 69.7 mg/l.

$$\frac{2 \text{ mg/kg/day} \times 70 \text{ kg}}{(2 \text{ liters} + (1.51 \times 0.0065) \times 1.0)} = 69.7 \text{ (or } \sim 70 \text{ mg/l)}$$

According to current guidelines, extrapolation from a LOAEL requires applying an uncertainty factor of 10. Furthermore, in light of the demonstrated bacterial mutagenicity of 2,4-DNP (Demerec, et al. 1951) and the suspected ability of the compound to induce chromosomal breaks in mammals (Mitra, and Manna, 1971), an additional uncertainty factor of 100 must be used in the criterion formulation.

The suggested water criterion for 2,4-DNP is, therefore:

$$\frac{70 \text{ mg/l}}{10 \times 100} = 70 \text{ } \mu\text{g/l}$$

If exposure is assumed to result from the consumption of contaminated fish or shellfish only, the criterion is 14.3 mg/l.

The available data are insufficient to enable calculation of water criterion levels for the remaining dinitrophenol isomers. For the present, it seems reasonable to assume that the 2,4-dinitrophenol criterion would be appropriate for the other isomers.

Chronic mammalian toxicology data for the trinitrophenols are absent from the literature. An outbreak of microscopic hematuria among shipboard U.S. Navy personnel exposed to 2,4,6-trinitrophenol in drinking water has been reported, however. It is not possible to precisely estimate either the 2,4,6-trinitrophenol water level or duration of exposure required for the development of hematuria. Consequently, criteria for trinitrophenol cannot be derived.

Although 4,6-dinitro-o-cresol (DNOC) is considered a cumulative poison in humans, probably as a result of slow metabolism and inefficient excretion, true chronic or subacute effects have never been reported in either humans or experimental animals. Since DNOC is not a cumulative poison in experimental animals, extrapolation to humans from long-term animal studies is of questionable value.

The no-observable-effect-level (NOEL) for DNOC respiratory exposure in humans has been reported as 0.2 mg/m³ air (NIOSH, 1978). NIOSH (1978) has, in fact, recommended that the current federal workplace environmental limit of 0.23 mg/m³ be retained, based on the available data.

It is possible to calculate the anticipated daily exposure of a 70 kg human male exposed to 0.2 mg/m³ for an eight-hour period. If one assumes the average minute volume is 28.6 l of air/minute (NIOSH, 1978) the anticipated daily exposure is 39 µg/kg/day. If the no-observed-effect-levels (NOELS) are calculated from long-term experimental animal studies, considerably higher values are obtained. The NOEL from respiratory exposure to DNOCs therefore is used as a basis for the water quality criterion.

If one assumes that absorption of DNOC across the respiratory tract is identical to gastrointestinal absorption, and that a 70 kg human consumes 2 l of water daily and 6.5 g of contaminated fish having a BCF of 5.49, the following calculation based on the NIOSH recommendation indicates the maximum allowable levels of DNOC in drinking water:

$$39 \text{ } \mu\text{g/kg/day} \times 70 \text{ kg} = 2.73 \text{ mg/day}$$
$$\frac{2.73 \text{ mg/day}}{2 \text{ l} + (5.49 \times 0.0065) \times 1.0} = 1.34 \text{ mg/l}$$

In view of the lack of data indicating chronic effects and the existence of a very recent federal guideline for human exposure, an uncertainty factor of 100 is chosen for the protection of the general public. The suggested criterion for 4,6-dinitro-o-cresol is:

$$\frac{1.34 \text{ mg/l}}{100} = 13.4 \text{ } \mu\text{g/l}$$

If exposure is assumed to be from the consumption of contaminated fish and shellfish only, the criterion level is 765 µg/l.

Sufficient data is not available with which to derive defensible criteria for other dinitro-o-cresol isomers.

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