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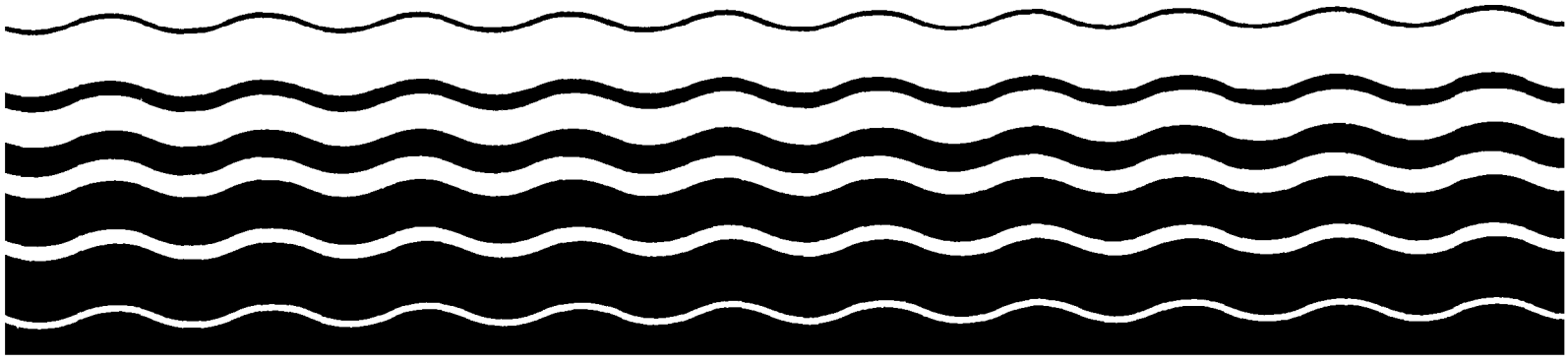
Ambient Water Quality Criteria for Dinitrotoluene



AMBIENT WATER QUALITY CRITERIA FOR

2,4-DINITROTOLUENE

Prepared By
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 satisifaction 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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DINITROTOLUENE

CRITERIA

Aquatic Life

The available data for dinitrotoluenes indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 330 and 230 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dinitrotoluenes indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 590 µg/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 dinitrotoluenes to sensitive saltwater aquatic life but a decrease in algal cell numbers occurs at concentrations as low as 370 µg/l.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of 2,4-dinitrotoluene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentration should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6} and 10^{-7}. The corresponding recommended criteria are 1.1 µg/l, 0.11 µg/l, and 0.011 µg/l, respectively.

If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 91 µg/l, 91.1 µg/l, and 0.91 µg/l, respectively.

INTRODUCTION

Dinitrotoluene (DNT) is an ingredient of explosives for commercial and military use because of its waterproofing action and explosive potential. Use is also made of DNT as a chemical stabilizer in the manufacture of smokeless powder.

DNT is produced by nitration of toluene to nitrotoluene to dinitrotoluene in a nitric and sulfuric acid solution (Lopez, 1977). The production of DNT is expected to increase yearly at a rate of 20 to 25 percent (Sittig, 1974). There are six isomers of dinitrotoluene, with the 2,4-isomer being the most important (Snell and Ettre, 1971). Often this isomer alone is referred to as DNT (Manufacturing Chemists Assoc., 1966) or dinitrotoluol (Sax, 1963).

Nitration of o-nitrotoluene yields mostly 2,4- and 2,6-dinitrotoluene, $CH_3C_6H_3(NO_2)_2$, in the ratio of about 65:35 (Wiseman, 1972).

2,6-DNT has a melting point of 66°C, a density of 1.2833 at 111°C, and is soluble in alcohol (Weast, 1975). Additional chemical and physical properties of this compound are: a boiling point of 285°C (Maksimov, 1968); a molecular weight of 182.14 (Weast, 1977); and a log octanol/water partition coefficient of 2.05 (Tute, 1971). Table 1 lists some physiochemical constants for 2,4-dinitrotoluene.

Except for their tendency to decompose at elevated temperatures, dinitrotoluenes are relatively stable. At 250°C, commercial grades of dinitrotoluene decompose at non-sustaining rates. However, at approximately 280°C rapid self-sustaining decomposition

occurs. Dinitrotoluenes may burn safely if unconfined, but if confined may result in an explosion. Decomposition may occur at lower temperatures in the presence of impurities (Manufacturing Chemists Assoc., 1966). Because of the deactivating effect of the two nitro groups in dinitrotoluenes, the synthesis of trinitrotoluene (TNT) does not occur readily (Wiseman, 1972).

TABLE 1

Some Physicochemical Constants
of 2,4-Dinitrotoluene*

Property	Value
Molecular weight	182.14
Melting point	69.5-70.5°C
Boiling point	300°C (dec.)
Density	
d_4^{15}	1.521
d_4^{71}	1.321
Vapor density (air=1)	6.27
Vapor pressure at 25±2°C	1.4×10^{-4} torr
Refractive index (n_D)	1.442
Solubility, grams/liter	
Water, at 22°C	0.27
Ethanol, at 15°C	30.46
Diethyl ether, at 22°C	94
Carbon disulfide, at 17°C	21.9
Heat of fusion (H_f)	26.4 cal/gram
Log octanol/water partition coefficient (Calc. by method of Tute, 1971)	2.01

*Source: Kirk and Othmer, 1967; St. John, et al. 1975; Weast, 1978

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Aquatic Life Toxicology*

INTRODUCTION

The data base for dinitrotoluenes is limited but 2,3-dinitrotoluene appears to be up to two orders of magnitude more acutely toxic to freshwater fish and invertebrate species than 2,4-dinitrotoluene. The tested fish and invertebrate species are similarly sensitive to these two dinitrotoluenes.

Acute toxicity tests using static conditions have been conducted with 2,3-dinitrotoluene and the sheepshead minnow and the mysid shrimp. The LC_{50} and EC_{50} values range from 370 µg/l for algal cell numbers to 2,280 µg/l for the sheepshead minnow.

EFFECTS

Acute Toxicity

Forty-eight-hour EC_{50} values are available for Daphnia magna for both 2,3- and 2,4-dinitrotoluene and are 660 and 35,000 µg/l, respectively (Table 1). The 96-hour LC_{50} for the fathead minnow and 2,4-dinitrotoluene is 31,000 µg/l (Table 1), and the 96-hour LC_{50} for the more toxic 2,3-dinitrotoluene and the bluegill is 330 µg/l.

The 96-hour LC_{50} values for the saltwater mysid shrimp and sheepshead minnow and 2,3-dinitrotoluene are 590 and 2,280 µg/l, respectively.

Chronic Toxicity

The chronic value for 2,3-dinitrotoluene, derived from an embryo-larval test with the fathead minnow, is 230 µg/l (Table 2) and is based on survival of these life stages (U.S. EPA, 1978). No acute-chronic ratio is calculable in the absence of a 96-hour LC_{50} for this fish species.

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*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.

No chronic toxicity data are available for any dinitrotoluene and saltwater organisms.

Plant Effects

Cell numbers of the freshwater alga, Selenastrum capricornutum, were reduced by 50 percent at a concentration of 2,3-dinitrotoluene of 1,370 µg/l (Table 3). A comparable inhibition in chlorophyll a occurred at a concentration of 1,620 µg/l.

A 50 percent reduction in cell numbers of the saltwater alga, Skeletonema costatum, occurred at a concentration of 370 µg/l 2,3-dinitrotoluene (Table 3). There was a 50 percent inhibition of chlorophyll a production at 400 µg/l.

Residues

No bioconcentration data are available for dinitrotoluenes and any aquatic organisms.

Summary

Few data are available for freshwater organisms but these data indicate that 2,3-dinitrotoluene is two orders of magnitude more toxic to fish and invertebrate species than is 2,4-dinitrotoluene. Also, the tested fish and invertebrate species appear to be of similar sensitivity. The 50 percent effect levels for 2,3-dinitrotoluene were within the range of 330 to 660 µg/l, and for 2,4-dinitrotoluene the range was 31,000 to 35,000 µg/l. A chronic value of 230 µg/l was calculated for the fathead minnow and 2,3-dinitrotoluene. The results of an algal assay with Selenastrum capricornutum and 2,3-dinitrotoluene were 96-hour EC_{50} values of 1,370 and 1,620 µg/l for cell number and chlorophyll a reduction.

Saltwater species have only been tested with 2,3-dinitrotoluene; the 96-hour LC_{50} values for the mysid shrimp and the sheepshead minnow were

590 and 2,280 µg/l, respectively. The saltwater alga, Skeletonema costatum, was of similar sensitivity as the mysid shrimp, with 96-hour EC_{50} values of 370 and 400 µg/l.

CRITERIA

The available data for dinitrotoluenes indicate that acute and chronic toxicity to freshwater aquatic life occur at concentrations as low as 330 and 230 µg/l, respectively, and would occur at lower concentrations among species that are more sensitive than those tested.

The available data for dinitrotoluenes indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 590 µg/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 dinitrotoluenes to sensitive saltwater aquatic life but a decrease in algal cell numbers occurs at concentrations as low as 370 µg/l.

Table 1. Acute values for dinitrotoluenes

Species	Method*	Chemical	LC50/EC50 (µg/l)	Species Acute Value (µg/l)	Reference
		FRESHWATER SPECIES			
Cladoceran, *Daphnia magna*	S, U	2,3-dinitro-toluene	660	660	U.S. EPA, 1978
Cladoceran, *Daphnia magna*	S, U	2,4-dinitro-toluene	35,000	35,000	U.S. Army, 1976
Fathead minnow, *Pimephales promelas*	S, U	2,4-dinitro-toluene	31,000	31,000	U.S. Army, 1976
Bluegill, *Lepomis macrochirus*	S, U	2,3-dinitro-toluene	330	330	U.S. EPA, 1978
		SALTWATER SPECIES			
Mysid shrimp, *Mysidopsis bahia*	S, U	2,3-dinitro-toluene	590	590	U.S. EPA, 1978
Sheepshead minnow, *Cyprinodon variegatus*	S, U	2,3-dinitro-toluene	2,280	2,280	U.S. EPA, 1978

* S = static, U = unmeasured

No Final Acute Values are calculable since the minimum data base requirements are not met.

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

Species	Method*	Chemical	Limits (µg/l)	Value (µg/l)
	FRESHWATER SPECIES			
Fathead minnow, *Pimephales promelas*	E-L	2,3-dinitro-toluene	200-270	230

* E-L = embryo-larval

No acute-chronic ratio can be estimated since no acute test data are available for this species.

Table 3. Plant values for dinitrotoluenes (U.S. EPA, 1978)

Species	Chemical	Effect	Result (µg/l)
	FRESHWATER SPECIES		
Alga, *Selenastrum capricornutum*	2,3-dinitro-toluene	Cell numbers 96-hr EC50	1,370
Alga, *Selenastrum capricornutum*	2,3-dinitro-toluene	Chlorophyll *a* 96-hr EC50	1,620
	SALTWATER SPECIES		
Alga, *Skeletonema costatum*	2,3-dinitro-toluene	Cell numbers 96-hr EC50	370
Alga, *Skeletonema costatum*	2,3-dinitro-toluene	Chlorophyll *a* 96-hr EC50	400

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Mammalian Toxicology and Human Health Effects

INTRODUCTION

2,4-Dinitrotoluene (2,4-DNT) is a pale yellow crystalline solid that is widely used as a raw material for dyestuffs and for urethane polymers through a conversion to the corresponding diamine and then to diisocyanate (Kirk and Othmer, 1967). Some of its physical properties are presented in Table 1. It is commercially prepared in the United States by the direct dinitration of toluene. The process produces an 80:20 ratio of 2,4-:2,6-isomers, which on fractionation gives pure 2,4-DNT (Kirk and Othmer, 1967). Precise production figures for 2,4-DNT are not available; however, the U.S. International Trade Commission (1975) reported a combined production of 272,610,000 pounds for the 2,4- and 2,6-DNT isomers in 1975.

The name given by the Chemical Abstracts Service (1977) for this compound is 1-methyl-2,4-dinitrobenzene (CAS registry number 121-14-2). Other synonyms for 2,4-DNT include 2,4-dinitrotoluol and toluene-2,4-dinitro. 2,4-DNT has a moderate fire and explosion risk and it can be detonated only by a very strong initiator.

Aside from its use by the dye and polyurethane manufacturing industries, 2,4-DNT is used by the munition industry as a modifier for smokeless powders and, to a limited extent, as a gelatinizing and waterproofing agent in military and commercial explosive compositions (Hamilton and Hardy, 1974). 2,4-DNT is also used as a chemical intermediate in the production of toluene diisocyanate (TDI) which, in turn, is consumed in the production of flexible and

TABLE 1

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d_4^{71}	1.321
Vapor density (air=1)	6.27
Vapor pressure at 25±2°C	1.4×10^{-4} torr
Refractive index (n_D)	1.442
Solubility, grams/liter	
Water, at 22°C	0.27
Ethanol, at 15°C	30.46
Diethyl ether, at 22°C	94
Carbon disulfide, at 17°C	21.9
Heat of fusion (H_f)	26.4 cal/gram
Log octanol/water partition coefficient (Calc. by method of Tute, 1971)	2.01

*Source: Kirk and Othmer, 1967; St. John, et al. 1975; Weast, 1978

rigid polyurethane foams and elastomers. Most TDI producers, however, use toluene as the starting material, generating 2,4-DNT as a captive intermediate (Kirk and Othmer, 1967).

The potential risk of exposure to 2,4-DNT is greatest for workers in the dye and explosives industries and at chemical plants producing TDI. 2,4-DNT encountered chiefly as a major component in the wastewater from munitions industries. The general population may experience exposure as a result of this discharge of 2,4-DNT into rivers and streams from munition plants (National Cancer Institute (NCI), 1978). Aromatic nitro compounds are one of several classes of chemicals thought to contribute to the increased cancer risk in dye and explosive manufacturing industries (Wynder, et al. 1963). The structural relationship of 2,4-DNT to the known carcinogen, 2,4-toluenediamine (2,4-TDA), is also a factor in its selection for testing as a possible carcinogen (NCI, 1978).

The usual methods of identification and quantitative determination of 2,4-DNT include spot tests (Ames and Yallop, 1966), colorimetry (Goldman and Jacobs, 1953), chromatographic methods such as thin layer chromatography (Yoshida, et al. 1967), gas chromatography (Krzymien and Elias, 1975; Pella, 1976; Fukuda, et al. 1977), and high pressure liquid chromatography (HPLC) (Walsch, et al. 1973; Doali and Juhasz, 1974; Stanford, 1977; National Institute for Occupational Safety and Health (NIOSH), 1978), and spectroscopic methods such as infrared (Priestera, et al. 1960) or ultraviolet spectrophotometry (Conduit, 1959), nuclear magnetic resonance spectrometry (Gehring and Reddy, 1968), mass spectrometry (Murrmann, et al. 1971; Plimmer and Klingebiel, 1974; Zitrin and

Yinon, 1976) and isotope dilution analysis (St. John, et al. 1975, 1976). In many other instances where the residues of explosives needed to be identified after an explosion, special wet chemical separation techniques were used (Hoffman and Byall, 1974; Jenkins and Yallop, 1970; Fukuda, et al. 1977).

EXPOSURE

Ingestion from Water

2,4-DNT has limited solubility (270 mg/l at 22°C) in water. Possible sources of 2,4-DNT in the aqueous environment, either surface water, ground water or drinking water, include the dumping of chemical wastes and accidental loss during transfer and transport.

Dinitrotoluene waste products are dumped into surface water or sewage by manufacturing industries that make dyes, isocyanates, polyurethanes, and munitions. The occurrence of organic micropollutants due to the dumping of aromatic nitro and amino compounds in river water has been reported by Meijers and Van der Leer (1976). The pollution of the Rhine and Maas Rivers in the Netherlands by these aromatics and oils was examined by extracting water samples in hexane followed by analysis of the extracts by gas chromatograph/mass spectrometry (GC/MS). The results showed that the Rhine is heavily polluted by oil, a number of aromatic hydrocarbons, aromatic amines and aromatic nitro compounds including 2,4-DNT. The Maas River, however, is much less polluted by these substances with the exception of oil.

The second source of water contamination by 2,4-DNT develops when the chemical is accidentally spilled during the process of

transfer and/or transportation. No specific incident of this type has been reported in the literature, however.

The ability of microorganisms to degrade 2,4-DNT and related compounds has been studied by a number of investigators (Schott, et al. 1943; Ruchhoft, et al. 1945; Ruchhoft and Norris, 1946; Rogovskaya, 1951; Nason, 1956; U.S. Army, 1970,1971; Osmon and Klausmier, 1972; Walsh, et al. 1973; Nay, 1974; Traxler, et al. 1974; Won, et al. 1974; McCormick, et al. 1976; Parrish, 1977). Biotransformation of 2,4-DNT does occur but its frequency is much lower than the equivalent activity for 2,4,6-trinitrotoluene (2,4,6-TNT). The influence of aromatic nitrated hydrocarbons including 2,4-DNT, on the activated sludge process has been extensively studied (Bogatyrev, 1973; Matsui, et al. 1975; Roth and Murphy, 1978). At concentrations of 50 mg/l of nitro-aromatics, there was no effect on the activated sludge process.

Ingestion from Food

The likelihood of 2,4-DNT existing in food is minimal, since it is not used as a pesticide or herbicide. There is no report in the literature, however, on the toxic effect of 2,4-DNT in humans due to ingestion from food.

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 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 2,4-dinitrotoluene, but the equation "Log BCF = (0.85 Log P) - 0.70" can be used (Veith, et al. 1979) to estimate the BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). Based on a measured log P value of 1.98 (Hansch and Leo, 1979), the steady-state bioconcentration factor for 2,4-dinitrotoluene is estimated to be 9.62. An adjustment factor of 3.0/7.6 = 0.395 can be 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. Thus, the weighted average bioconcentration factor for 2,4-dinitrotoluene and the edible portion of all aquatic organisms consumed by Americans is calculated to be 9.62 x 0.395 = 3.8.

Inhalation

An estimate of the number of individuals involved in the manufacture of 2,4-DNT in the United States is not available at present. But the U.S. International Trade Commission (1975) reports a combined production of 272,610,000 pounds for the 2,4-and 2,6-DNT isomers in 1975. Since DNT is produced in such large quantities, a considerable proportion of the population may be at risk.

Inhalation has been reported to be one of the major routes of exposure to 2,4-DNT in either the particulate or vapor state. The effects of inhalation exposure to 2,4-DNT are a consequence of its capacity to produce anoxia due to the formation of methemoglobin (see Effects Section).

There are no data in the literature on the ambient atmospheric concentration of 2,4-DNT. Thus, it is not possible to estimate the extent of possible human exposure.

Dermal

Since 2,4-DNT is readily soluble in organic solvents such as alcohol, ether, etc., it penetrates the intact skin readily (Patty, 1958; Hamblin, 1963). From a survey of the literature (Toxic and Hazardous Industrial Chemicals Safety Manual, 1976; Key, et al. 1977; Proctor and Hughes, 1978), it is obvious that skin contact is another important route for 2,4-DNT absorption in plant workers. The quantitative data on the threshold doses for dermal absorption of 2,4-DNT are unavailable in the literature. However, the Occupational Safety and Health Administration (OSHA) recommends a threshold limit value (TLV) of 1.5 mg/m^3 of air including dermal exposure (American Conference of Governmental Industrial Hygienists

(ACGIH), 1978). This TLV was set by analogy to chemically similar nitro-aromatic compounds (ACGIH, 1974).

Because of the limited availability of data on the human exposure to 2,4-DNT, it is difficult to assess quantitatively the contribution of each route of exposure to the total dose; it is likely that the greatest contribution comes via inhalation, particularly in an occupational setting. The next most likely route is dermal and the least likely is ingestion.

PHARMACOKINETICS

Absorption, Distribution, and Excretion

2,4-DNT is absorbed mainly by inhalation of its vapor or by percutaneous aborption of its solution in organic solvents. Hodgson, et al. (1977) recently reported a study on the comparative absorption, distribution, and excretion of 2,4,6-TNT and isomers of DNT in rats. It was noticed that the ^{14}C ring-labeled nitrotoluenes were well absorbed after oral administration in the rat. The absorption was essentially complete in 24 hours with 60 to 90 percent of the dose being absorbed. The extent of absorption occurred in the following order: 2,4-DNT = 3,4-DNT $>$ 3,5-DNT = 2,4,6-TNT = 2,5-DNT $>$ 2,3-DNT = 2,6-DNT. The liver, kidneys and blood contained small amounts of radioactivity. The ratios of radioactivity (tissue:plasma) indicated a retention of ^{14}C in both the liver and kidneys, while negligible amounts of ^{14}C were found in the other tissues. No ^{14}C was recovered in the expired air; most of the absorbed radioactivity was eliminated in the urine. When ^{14}C-labeled nitrotoluenes were administered to bile duct-cannulated rats, 10.3 to 27.3 percent of the ^{14}C was recovered in the bile,

suggesting that biliary excretion is also an important elimination pathway. Thin layer chromatographic analysis of the urine from rats treated with 2,4,6-TNT or dinitrotoluene indicated extensive metabolism of the parent compounds. However, this study does not report the characterization of the metabolic products from dinitrotoluenes and 2,4,6-TNT.

In another study the distribution and excretion of tritium-labeled 2,4-dinitrotoluene (^{3}H-2,4-DNT) in the rat was examined (Mori, et al. 1977). Approximately 21.3 percent of the radioactivity was excreted in the feces on the first day after a single oral administration of ^{3}H-2,4-DNT. The amount of radioactivity excreted in the feces on the second and third days were 4.1 and 1.25 percent of the administered dose, respectively. About 13.5 percent of the radioactivity administered was excreted in the urine on the first day, but after the second day the urinary excretion of radioactivity occurred in only trace quantities. In all, about 47 percent of the radioactivity administered was excreted in the feces and urine during the first seven days following administration (see Table 2).

In the same experiment, relatively high amounts of radioactivity were found in adipose tissue, skin, and liver of the rats seven days after administration; the relative amounts of radioactivity remaining in other organs were not significant (Table 3). This investigation, utilizing the single oral administration of ^{3}H-2,4-DNT, suggests that 2,4-DNT remains in the liver, skin, and adipose tissue.

TABLE 2

Urinary and Fecal Excretion of Radioactivity,
Expressed as Percentages of Administered Radioactivity*

Day	Urine (%)	Feces (%)
1st	13.52±1.44	21.34±3.10
2nd	0.61±0.12	4.11±0.53
3rd	0.66±0.12	1.25±0.41
4th	0.48±0.18	0.78±0.12
5th	0.28±0.08	0.77±0.14
6th	0.19±0.09	0.84±0.21
7th	0.15±0.03	1.23±0.02

Values are indicated as means and deviations of three rats.

*Source: Mori, et al. 1977

TABLE 3

Remaining Radioactivity in the Tissues of Rat
Seven Days after Administration of ^{3}H-2,4-DNT*

Tissue	Radioactivity		
	dpm per 100 mg Tissue x 10^{3}	Total dpm x 10^{4}	% of Dose
Brain	0.93	1.19	0.03
Heart	0.99	0.49	0.01
Lung	1.14	1.12	0.03
Liver	1.98	17.23	0.40
Spleen	0.81	0.36	0.01
Pancreas	1.30	0.71	0.02
Kidney	0.98	1.77	0.04
Adrenal	2.11	0.03	trace
Stomach	0.80	0.60	0.01
Small intestine	0.99	4.56	0.10
Large intestine	1.02	0.84	0.02
Testis	0.85	1.98	0.04
Mesenteriolum	0.82	1.54	0.04
Adipose tissue	13.99	68.30	1.60
Skin	0.79	25.53	0.60

Mean of three rats given 50 mg of ^{3}H-2,4-DNT/kg p.o.
Weights of skin and adipose tissue were roughly calculated as:
skin = body weight x 1/25; adipose tissue = body weight x 1/40.

*Source: Mori, et al. 1977

Metabolism

No report has yet been published on the metabolic fate of 2,4-DNT in humans. Even the two studies (Hodgson, et al. 1977; Mori, et al. 1977) which describe the absorption, distribution, and excretion of 2,4-DNT in rats do not give details on the characterization of metabolites and metabolic pathways.

The isolation, identification and synthesis of biotransformation products derived from 2,4-DNT have been reported by McCormick, et al. (1978) from a detailed study on the microbial transformation of 2,4-DNT by Mucrosporium sp. (Strain QM 9651). The biotransformation products were identified by thin layer chromatography (using silica gel plates with fluorescent indicator to visualize the metabolites and developing in benzene-hexane 50:50 percent v/v solvent mixtures) and then were followed by GC/MS. The metabolites identified were 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,2'-dinitro-4,4'-azoxytoluene, 4,4'-dinitro-2,2'-azoxytoluene, and 4-acetamido-2-nitrotoluene; a third azoxy compound, believed to be a "mixed" type (i.e., 2,4'-azoxy or 4,2'-azoxy), was also isolated, but not identified. These authors present a scheme for the biotransformation of 2,4-DNT (Figure 1). Although no 2,4-toluenediamine (2,4-TDA) was detected in the present system, complete reduction of both nitro groups to amino groups has been reported in the biotransformation of 2,4-DNT by anaerobic bacterial systems (McCormick, et al. 1976); hence, 2,4-TDA is also included in Figure 1.

In a study of the microbial transformation of 2,4-DNT, 2,4,6-TNT and other nitroaromatic compounds by anaerobic bacterial

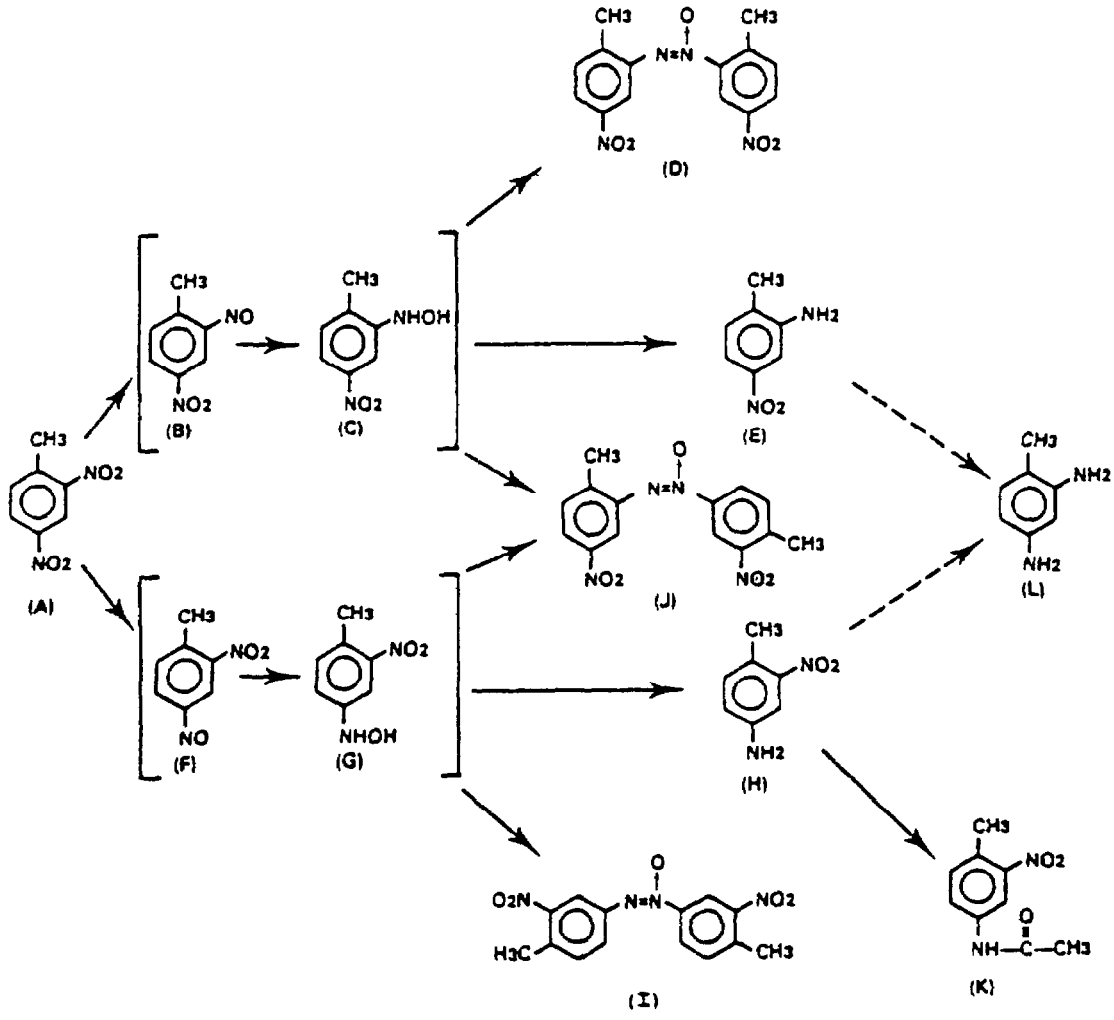

FIGURE 1

Proposed Pathways for the Formation of Biotransformation Products from 2,4-Dinitrotoluene (A)

Source: McCormick, et al. 1978

The hypothetical nitroso and hydroxylamino intermediates are enclosed in brackets. The potential formation of 2,4-toluenediamine (L) is indicated by dashed arrows.

(B) 2-Nitroso-4-nitrotoluene; (C) 2-Hydroxylamino-4-nitrotoluene; (D) 4,4'-Dinitro-2,2'-azoxytoluene; (E) 2-Amino-4-nitrotoluene; (F) 4-Nitroso-2-nitrotoluene; (G) 4-Hydroxylamino-2-nitrotoluene; (H) 4-Amino-2-nitrotoluene; (I) 2,2'-Dinitro-4,4'-azoxytoluene; (J) 4,2'-Dinitro-2,4'-azoxytoluene; (K) 4-Acetamido-2-nitrotoluene

systems (McCormick, et al. 1976), these compounds were reduced by hydrogen in the presence of enzyme preparations from Veillonella alkalescens. Consistent with the proposed reduction pathways, $R\text{-}NO_2 \xrightarrow{H_2} R\text{-}NO \xrightarrow{H_2} R\text{-}NHOH \xrightarrow{H_2} R\text{-}NH_2$, 3 moles of H_2 were utilized per mole of nitro group. From the rates of reduction of 40 mono-, di-, and trinitroaromatic compounds by Veillonella alkalescens, it was noticed that reactivity of the nitro group depended on other substituents and on the position of the nitro groups relative to these substituents. The order of reduction rate of nitro compounds is consistent with the "electronegativity rule" (Shikata and Tachi, 1938):

$$-NO_2 > -COOH > -CH_3 > -H > -OH > -NH_2$$

In the case of nitrotoluenes, the para nitro group was the most readily reduced, the 4-nitro position of 2,4-DNT being reduced first. The "nitro-reductase" activity of Veillonella alkalescens extracts was associated with protein fractions, one having some ferredoxin-like properties and the other possessing hydrogenase activity. The question of whether ferredoxin acts as a nonspecific reductase for nitroaromatic compounds remains unresolved.

Since the microbial transformation pathway of 2,4-DNT (McCormick, et al. 1978) is similar to that of 2,4,6-TNT (McCormick, et al. 1976), it can be assumed that these two compounds may behave similarly during biochemical transformation in animals and humans. Hence, it is reasonable to discuss a few studies on the metabolism of 2,4,6-TNT in animals and humans in this context.

The explosive 2,4,6-TNT has been extensively investigated because of the toxic symptoms which it produces in people engaged

in its manufacture (Palmer, et al. 1943; Schwartz, 1944; Crawford, 1954; Goodwin, 1972; Djerassi and Vitany, 1975; Morton, et al. 1976). It is generally agreed that its toxicity is due to its metabolic products (Won, et al. 1974, 1976; Carpenter, et al. 1978). Earlier studies (White and Hay, 1901; Moore, 1918; Schereschewsky, 1918; Voegtlin, et al. 1920) have shown that the urine of 2,4,6-TNT workers and of experimental animals receiving 2,4,6-TNT orally or by injection contained 2,2',6,6'-tetranitro-4,4'-azoxytoluene and 2- or 4-aminodinitrotoluene. The investigations of Channon, et al. (1944) showed that rabbits, when given small oral doses of 2,4,6-TNT, excreted 2- and 4-aminodinitrotoluenes and 4-hydroxylamino-2,6-dinitrotoluene. Of the two amino compounds excreted, the 4-amino-2,6-dinitrotoluene was found in larger quantities and the 4-hydroxylamino-2,6-dinitrotoluene was obviously an intermediate in the reduction of 2,4,6-TNT to the corresponding amino compound. The 4-amino-2,6-dinitrotoluene was also formed when 2,4,6-TNT was incubated with an acetone extract of pig liver (Bueding and Jolliffe, 1946). When administered to pigs, some 24 to 30 percent of the 2,4,6-TNT appears in the urine as compounds containing a diazotizable amino group. In man, 2,4,6-TNT appears to be converted to the same metabolites as in the rabbit (Williams, 1959). Dale (1921) showed that 2,2',6,6'-tetranitro-4,4'-azoxytoluene could be isolated from the urine of 2,4,6-TNT workers, a fact which indicates that 2,4,6-TNT is reduced in man to 4-hydroxylamino-2,6-dinitrotoluene. Lemberg and Callaghan (1944) also detected the 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene in human urine. These authors stated that the qualitative and quantitative

distribution of 2,4,6-TNT metabolites in human urine is similar to that found in rabbit urine. A scheme for the biotransformation of 2,4,6-TNT is presented in Figure 2. It is interesting to note that no study in the literature reports the formation of 2,4,6-triaminotoluene as a metabolic product of 2,4,6-TNT, though such a possibility cannot be ruled out.

Thus, by analogy of metabolism of 2,4,6-TNT to that of 2,4-DNT (compare Figures 1 and 2), one might expect most of the products presented in Figure 1 to be present in the urine of humans and animals exposed to 2,4-DNT. Most of these metabolites are either toxic (Fairchild, et al. 1977) or suspected carcinogens (Christensen, et al. 1976).

EFFECTS

Acute, Subacute, and Chronic Toxicity

Acute toxic effects of 2,4-DNT include methemoglobinemia followed by cyanosis. The inhalation of the fumes or dust, the ingestion of the compound, or the absorption by the skin through contact of 2,4-DNT all cause a chemical change of the blood oxyhemoglobin into methemoglobin (via oxidation of Fe(II) to Fe(III)). The onset of symptoms of methemoglobinemia due to the absorption of 2,4-DNT is often insidious and may be delayed up to four hours; headache is commonly the first symptom and may become quite intense as the severity of methemoglobinemia progresses. The following symptoms have been reported as a result of varying doses of 2,4-DNT: vertigo, fatigue, dizziness, weakness, nausea, vomiting, dyspnea, drowsiness, arthralgia, insomnia, tremor, paralysis, unconsciousness, chest pain, shortness of breath, palpitation (rapid throbbing

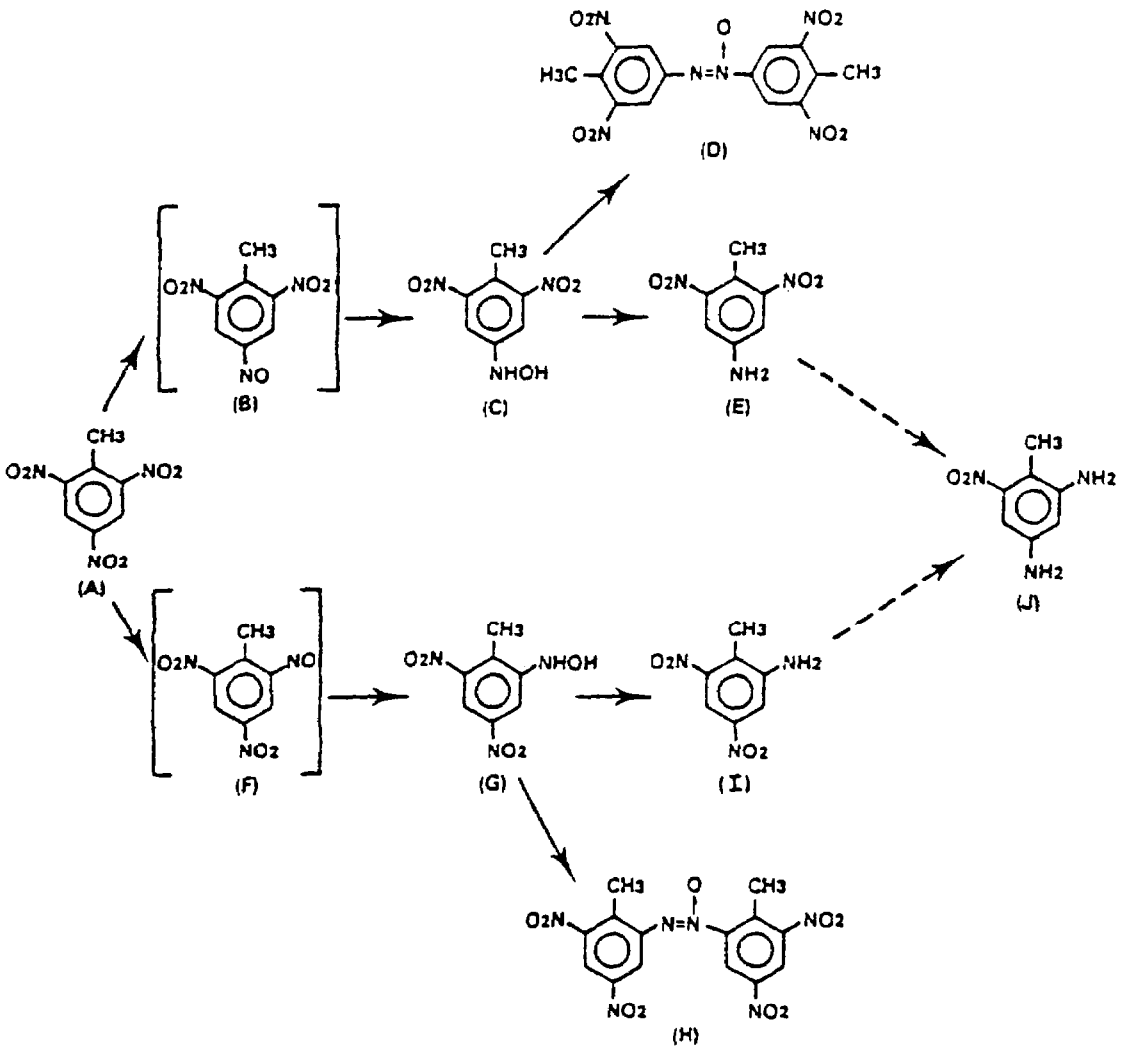

FIGURE 2

Proposed Pathways for the Formation of Biotransformation Products from 2,4,6-Trinitrotoluene (A) The hypothetical nitroso intermediates are enclosed in brackets. The potential formation of 2,4-Diamino-6-nitrotoluene (J) is indicated by dashed arrows.

(B) 4-Nitroso-2,6-dinitrotoluene; (C) 4-Hydroxylamino-2,6-dinitrotoluene; (D) 2,2',6,6'-Tetranitro-4,4'-azoxytoluene; (E) 4-Amino-2,6-dinitrotoluene; (F) 2-Nitroso-4,6-dinitrotoluene; (G) 2-Hydroxylamino-4,6-dinitrotoluene; (H) 4,4',6,6'-Tetranitro-2,2'-azoxytoluene; (I) 2-Amino-4,6-dinitrotoluene

Source: Williams, 1959; Won, et al. 1974

of heart), anorexia (lack of appetite), and loss of weight (Koelsch, 1917; Von Oettingen, 1941; Mangelsdorff, 1952, 1956; Hamblin, 1963; Toxic and Hazardous Industrial Chemicals Safety Manual, 1976; Key, et al. 1977; Proctor and Hughes, 1978). 2,4-DNT also produces Heinz bodies (granules in red blood cells due to damage of the hemoglobin molecules) in the cat (Bredow and Jung, 1942). Human subjects are similarly susceptible, and workers handling compounds such as nitrobenzenes, nitrotoluenes and phenylhydrazines occasionally exhibit Heinz bodies in their blood (Hughes and Treon, 1954; De Bruin, 1976).

Inactivation of hemoglobin due to 2,4-DNT and related compounds has been noted by Vasilenko, et al. (1972). These authors observed the transformation of hemoglobin into methemoglobin, nitrosylhemoglobin, and sulfhemoglobin when rats received 0.1 to 0.2 LD_{50} of 2,4-DNT orally for a period of 30 days. An increase in the levels of methemoglobin and sulfhemoglobin was accompanied by a decrease in oxyhemoglobin, but the total level of hemoglobin remained unchanged.

Methemoglobin formation of nitrotoluenes in relation to the number and positioning of nitro groups was studied by Kovalenko (1973). When administered orally at doses corresponding to 0.1 to 0.2 LD_{50} values to rats for one to three months, the hemotoxicity of the nitrotoluenes decreased in the order: trinitrotoluene > dinitrotoluene > m-nitrotoluene, p-nitrotoluene > o-nitrotoluene.

Cyanosis due to the absorption of 2,4-DNT occurs when the methemoglobin concentration of the blood is 15 percent or more. The symptoms observed include blueness in the lips, the nose, and the

earlobes. The individual usually feels well, has no complaints, and insists that nothing is wrong until the methemoglobin concentration approaches approximately 40 percent, when there usually is weakness and dizziness; at levels of about 70 percent methemoglobin there may be ataxia, dyspnea on mild exertion, tachycardia, nausea, vomiting, and drowsiness (Hamblin, 1963). With an increase in ambient temperature, and an associated increase in vapor pressure there is an increased susceptibility to cyanosis due to higher exposure levels of 2,4-DNT (Linch, 1974).

Some earlier studies provide useful information on the toxicity of 2,4-DNT. Animal experiments reported by White, et al. (1902) indicate that 2,4-DNT is comparatively less toxic than 1,3-dinitrobenzene. They found that cats may tolerate the repeated oral administration of 2 or 4 ml of a 1 percent solution of 2,4 DNT in cod liver oil, until a total of 24 ml has been given, without any apparent toxic effect. Similarly, Zieger (1913) observed no toxic effects due to the inhalation of 2,4 DNT vapors by cats, whereas Kuhls (1908) found that the subcutaneous injection of cats with 0.05 to 0.5 g of 2,4-DNT dissolved in mineral oil resulted in death within 2 to 23 days. Dambleff (1908) reported no indication of percutaneous toxicity; similarly, Kuhls (1908) observed no toxic effects in cats resulting from the cutaneous administration of 0.3 g/kg body weight, while Zieger (1913) found that two doses of 5 g each were fatal to cats eight hours after administration.

A list of the toxic doses for a number of animal species is presented in Table 4. The rat oral LD_{50} values listed in Table 4 are comparable to those of nitrobenzene and 2,6-DNT. The mouse

TABLE 4

Acute Toxic Levels of 2,4-Dinitrotoluene
for Different Species*

Species	Route	Toxicity	Dose (mg/kg)
Rat	Oral	LD_{50}	268
Mouse	Oral	LD_{50}	1,625
Cat	Oral	MLD	27
	s.c.	LDLo	50-500

s.c. - subcutaneous; LDLo - lowest published lethal dose; LD_{50} - lethal dose 50 percent kill; MLD - minimum lethal dose

*Source: Spector, 1956; Fairchild, et al. 1977; Vernot, et al. 1977

oral toxicity follows the order: aniline > 1,3,5-trinitrobenzene > 2,6-DNT > 3-nitrotoluene = 4-nitrotoluene = 2,5-DNT > 2,4-DNT > 2-nitrotoluene.

With regard to the human toxicity of 2,4-DNT, toxic effects may only occasionally be observed from the handling of the pure material. In addition to the complaints discussed above due to methemoglobinemia, more severe cases involving dyspnea, dizziness, sleepiness, and pain in the joints (especially in the knee) have been reported (Perkins, 1919). Perkins (1919) also pointed out that during the purification of the crude 2,4-DNT cakes, toxic vapors may be inhaled and the material may be sufficiently absorbed through the skin to cause toxic effects. Floret (1929) reported a severe case of 2,4-DNT poisoning, in which the patient (a plant worker) suffered from severe cyanosis and complained later of headache, palpitation of heart, tightness in the chest, insomnia and lack of appetite. Upon examination, medical findings indicated tremors of varying intensity in the hands, arms, head, extended fingers and tongue, nystagmus, and impaired reflexes. Lewin (1921) stated that exposure to 2,4-DNT may result in temporary visual disturbances.

The metabolic disturbances in workers exposed to 2,4-DNT were extensively studied by McGee, et al. (1942). A number of signs and symptoms of chemical intoxication appeared in a large group of inexperienced workmen following their introduction into military screening and coating houses which use 2,4-DNT. The chief symptoms of a group of 154 workers so exposed were an unpleasant metallic taste, weakness, headache, loss of appetite, and dizziness. Two-thirds of the men in the group selected for study had these

complaints at one time or another during the 12-month exposure period. One-half of the group developed clinical signs of intoxication, chiefly pallor, cyanosis and low-grade anemia. Jaundice was observed in two patients. No instances of permanent physical impairment were found. The symptoms described by these workers are presented in Table 5; Table 6 presents the chief findings from clinical examinations of these workers.

There is no report in the literature that discusses the mechanism of toxic action of 2,4-DNT per se. Usually its toxic action is presented along with other structurally related aromatic nitro and amino compounds. Most of the aromatic nitro and amino compounds are not in themselves cyanogenic, but oxidation-reduction enzyme systems promote biotransformation to active cyanogenic derivatives that arise from either reduction of the nitro group or oxidation of the amine. Most of the aromatic nitro and amino compounds that have been investigated, regardless of species, including man, come to a point of equilibrium,

$$\text{Methemoglobin} \rightleftharpoons \text{Hemoglobin},$$

beyond which, in spite of further dosage, no appreciable increase in methemoglobin concentration can be obtained (Hamblin, 1963). Bodansky (1951) also points out that there normally exists an equilibrium in blood between hemoglobin and methemoglobin, which is usually shifted far to the right. He believes that this shift is regulated by various oxidizing and reducing substances produced during in vivo metabolism, and that such a concept helps to explain the difference in degree of methemoglobin formation in various species, as well as the differing rates of reduction of methemoglobin

TABLE 5

Symptoms Presented by 154 2,4-Dinitrotoluene Workers*

Symptom	Screening House Number of Workmen	Coating House and Air dry Number of Workmen	Total	
			Number	Percent
Unpleasant taste in mouth	62	34	96	62
Weakness	51	27	78	51
Headache	48	28	76	49
Inappetence	42	30	72	47
Dizziness	43	25	68	44
Nausea	39	18	57	37
Insomnia	37	20	57	37
Pain in extremities	26	14	40	26
Vomiting	22	13	35	23
Numbness and tingling	18	11	29	19
Loss of weight (5 pounds or more)	7	3	10	6.5
Diarrhea	3	5	8	5.2

*Source: McGee, et al. 1942

TABLE 6

Clinical Findings in 154 2,4-Dinitrotoluene Workers*

Finding	Screening House (Number of workmen)	Coating House (Number of workmen)	Total	Percent
Pallor	40	15	55	36
Cyanosis	38	14	52	34
Anemia	28	8	36	23
Leucocytosis	12	7	19	12
Hypotension	8	1	9	5.8
Skin rash	2	4	6	3.9
Leukopenia	2	3	5	3.2
Hepatitis and Jaundice	1	1	2	1.4

*Source: McGee, et al. 1942

to hemoglobin. Methemoglobin-forming capacity in the cat of some aromatic nitro and amino compounds including 2,4-DNT are presented in Table 7.

From a ten-year study on the biological monitoring for industrial exposure to cyanogenic aromatic nitro and amino compounds, Linch (1974) establishes a reasonably good relationship between causative agent structure and biochemical hazard in order to rank the relative hazard of these chemicals. In this study, dinitrotoluenes are ranked No. 12 (1 most potent, 13 least potent) indicating that 2,4-DNT does not produce cyanosis as rapidly as other cyanogenic aromatic nitro and amino compounds. From the similarities of its toxic effects with other structurally related aromatic nitro compounds, and also from the available information of its metabolic pathway (as presented in Figure 1), a possible cyanosis mechanism for 2,4-DNT is presented in Figure 3.

Subacute toxicity of 2,4-DNT in dogs, rats, and mice was studied by Ellis, et al. (1976). 2,4-DNT was given orally to dogs in daily doses of 1, 5, or 25 mg/kg and to rats and mice in feed as 0.07, 0.2, or 0.7 percent of their diet for 13 weeks. Toxic effects in the dogs and rats included inhibition of muscular coordination in the hind legs, rigidity in extension of the hind legs, decreased appetite, and weight loss. Only the appetite and weight effects were observed in mice. The highest doses were lethal to some animals in all three species, while the lowest doses produced no toxic effects. All species showed methemoglobinemia and anemia with reticulocytosis. Characteristic tissue lesions were extramedullary hematopoeisis in the spleen and liver, gliosis and demyelination

TABLE 7

Methemoglobin-forming Capacity of Some Aromatic Nitro and Amino Compounds in Cat*

Compound	Molar ratio[a]
Nitrobenzene	0.86
1,3-Dinitrobenzene	7.1
1,3,5-Trinitrobenzene	4.8
2-Nitrotoluene	0.05
3-Nitrotoluene	0.04
4-Nitrotoluene	Very slight
2,4-Dinitrotoluene	1.4
2,6-Dinitrotoluene	0.55
2,4,6-Trinitrotoluene	1.7
Aniline	2.5 (2.7)
Phenylhydroxylamine	34.0
3-Aminonitrobenzene	3.0
1,3-Diaminobenzene	1.4
Nitrosobenzene	8.6

*Source: Hamblin, 1963; De Bruin, 1976

[a]Molar ratio of methemoglobin formed to dose of test compound

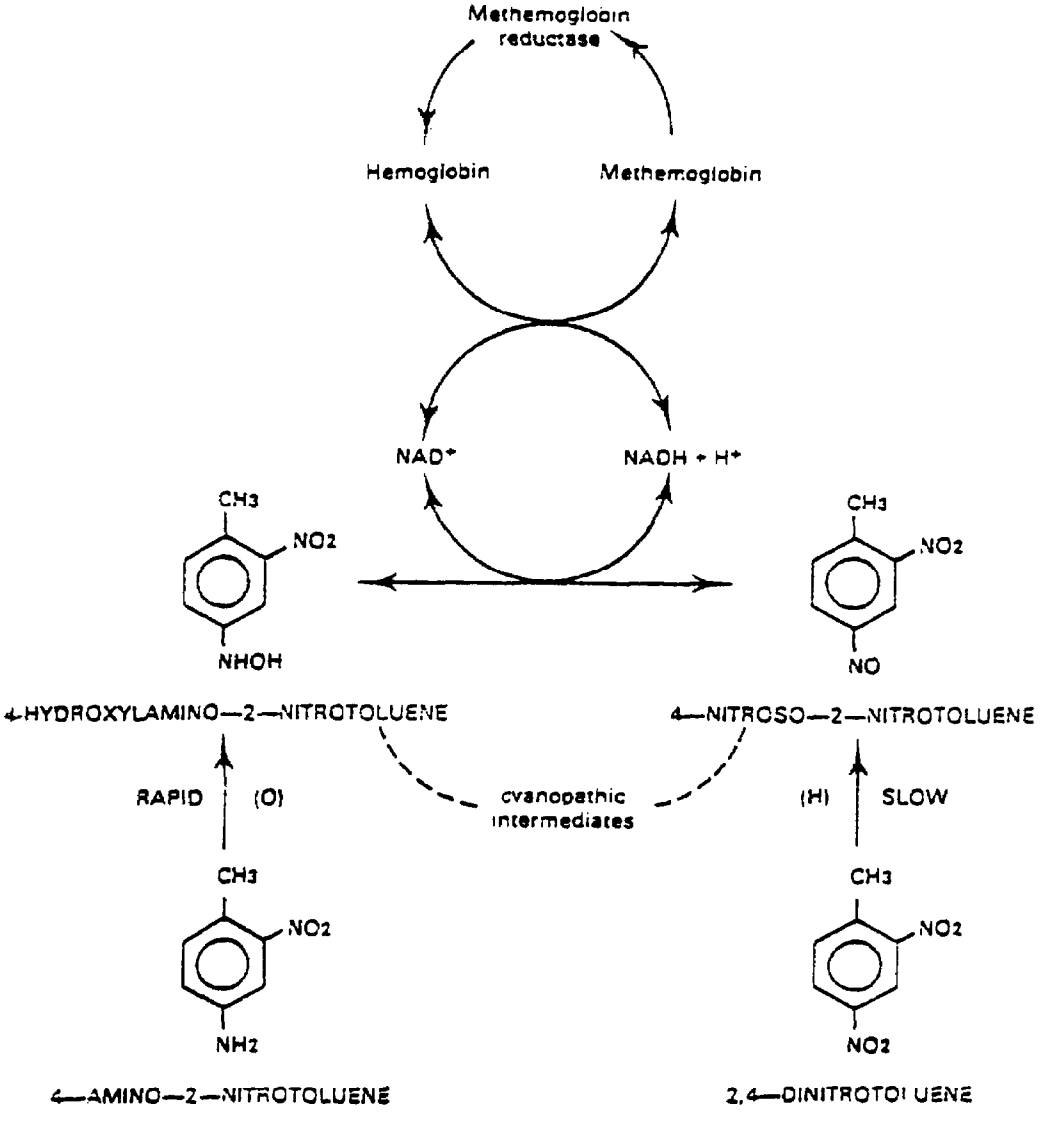

FIGURE 3

Suggested Metabolic Pathway for Cyanosis by 2,4-Dinitrotoluene and for 4-Amino-2-nitrotoluene Based upon Data from Related Compounds.

in the brain, and atrophy with aspermatogenesis in the testes. 2,6-DNT tested similarly in dogs (Ellis, et al. 1976) at 4, 20, or 100 mg/kg/day and in rats and mice at 0.01, 0.05, and 0.25 percent in their diet, produced similar effects. It was concluded that the primary subacute toxic effects of 2,4- and 2,6-DNT are seen in the red cells, nervous system, and testes.

Chronic exposure of 2,4-DNT may produce liver damage, jaundice, and reversible anemia due to blood damage (Linch, 1974; Key, et al. 1977; Proctor and Hughes, 1978). Liver injury may be more common than cyanosis, especially if the diet is deficient in protein (von Oettingen, 1941; Gleason, et al. 1969). Kovalenko (1973) reports that the chronic exposure of 2,4-DNT in rats caused anemia accompanied by reticulocytosis, a decrease in the level of sulfhydryl groups, and an increase in that of fibrinogen in the blood.

Influence of diet on the chronic toxicity of 2,4-DNT in mice was studied by Clayton and Baumann (1944). Mice fed with 2,4-DNT grew better on diets high in fat than those fed on other diets. Those animals maintained on diets low in fat and fed 2,4-DNT showed a retardation in the rate of growth, and many died within five weeks. Mice raised to maturity on the low fat diet or on a procarcinogenic diet were less resistant to toxicity from parenteral 2,4-DNT than mice raised on the other diets.

From another study on the effect of fat and calories on the resistance of mice to chronic toxicity of 2,4-DNT, Clayton and Baumann (1948) observed that mice ingesting 2,4-DNT grew less and died faster when fed a diet moderately low in fat (0.46 percent) than when fed the same amount of 2,4-DNT per calorie in diets

containing 5 or 30 percent added fat. Fat likewise appeared to minimize the toxic effects of 2,4-DNT in rats. When the effects of a low calorie intake are corrected for, 2,4-DNT per se retarded growth only slightly. Clayton and Baumann (1948) noted that many different fats and oils appeared equally active in minimizing the toxic effects of 2,4-DNT.

The effect of diet on the susceptibility of the rat to chronic poisoning by 2,4-DNT was also studied in detail by Shils and Goldwater (1953). A high intake of fat, in the form of corn oil, was found to be definitely beneficial with respect to the survival of rats subsisting on a low-protein intake and receiving 2,4-DNT parenterally. Increased amounts of protein with a low fat diet prevented death, regardless of the mode of 2,4-DNT administration.

Synergism and/or Antagonism

Ingestion of alcohol has a synergistic effect on the toxicity of 2,4-DNT. Friedlander (1900) discussed a patient who exhibited acute confusion and retrograde amnesia after exposure to 2,4-DNT and drinking a small amount of beer. This synergistic effect of alcohol on the toxicity of 2,4-DNT was also noted by McGee, et al. (1942). Of the group of 154 male workers exposed to 2,4-DNT in military screening and coating houses, 23 showed a reduced tolerance for alcohol and 31 stated that their toxic symptoms had been aggravated by ingesting alcohol. Some workers reported that they had found it impossible to drink any alcoholic beverage within two to three hours after finishing a shift without experiencing reactions such as substernal pressure, precardial palpitation, fullness in the head, and severe acute illness.

The ingestion of alcohol normally causes increased susceptibility to cyanosis; thus, alcohol in any form should never be administered to a victim of 2,4-DNT poisoning. Furthermore, since the body eliminates 2,4-DNT rather slowly, abstention from alcoholic beverages should be practiced for several days after 2,4-DNT exposure (Von Oettingen, 1941; Key, et al. 1977; Proctor and Hughes 1978).

Teratogenicity

No studies were found in the literature which addressed the teratogenicity of 2,4-DNT or the other isomers of dinitrotoluene.

Mutagenicity

The data available in the literature on the mutagenicity of 2,4-DNT are limited and rather confusing. Studies by Hodgson, et al. (1976) show some positive results. The mutagenic effect of 2,4-DNT on germinal cells was studied by these authors using the dominant lethal assay on rats fed a diet containing 2,4-DNT for 13 weeks. Females mated to males treated with 0.2 percent 2,4-DNT showed a significant increase in the number of dead implants/total implants over control animals.

Hodgson, et al. (1976 abstract) also screened for somatic cell mutation effects by cytogenetic analysis of lymphocyte and kidney cultures derived from rats fed 0.2 percent of 2,4-DNT for 19 weeks. No increase in the frequency of translocations or chromatid breaks was observed in either the lymphocyte or kidney cultures. However, significant increases in the frequency of chromatid gaps were observed in kidney cultures after five weeks and in lymphocytes at 19 weeks. This would suggest that 2,4-DNT has a potential for inducing

damage in somatic cells. In vitro studies using the CHO-KI test system were negative. On the other hand, microbial tests using Salmonella typhimurium TA 1535 indicated that 2,4-DNT is capable of producing base-pair mutations. Details of the methodology used were not available.

There are two other reports in the literature (Simmon, et al. 1977; Cotruvo, et al. 1977) which discuss the mutagenic effects of products from ozonation or chlorination reactions of 2,4-DNT and other related di- and trinitrotoluenes. In the study by Simmon, et al. (1977), a number of compounds present in waste water from munitions plants were examined before and after ozonation or chlorination to determine whether mutagenic activity was affected by the treatment. Test materials included 1,3-dinitrobenzene; 2,4-DNT; 3,5-DNT, 2,4,6-TNT; 2,4,6-TNT production waste water; hexahydro-1,3,5-trinitro-s-triazine (RDX); octahydro-1,3,5,7-tetranitro-s-tetrazine (HMX); components of photolyzed 2,4,6-TNT; pentaerythritol tetranitrate, and trinitroresorcinol. The in vitro mutagenic assays used were the Salmonella/microsome assay (Ames, et al. 1973a,b) with strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 and mitotic recombination in the yeast, Saccharomyces cerevisiae D3. A metabolic activation system using the postmitochondrial supernatant fraction of liver from rats, pretreated with Aroclor 1254, was included in each assay procedure. Under these conditions, neither ozonation nor chlorination significantly altered the mutagenic activity of the nitro aromatic materials tested, including 2,4-DNT.

In the investigation of mutagenicity of products of ozonation in water by Cotruvo, et al. (1977), compounds such as 2,4-DNT, phenol, hydroquinone and nitrilotriacetic acid were found to give anomalous results in Saccharomyces after ozonation. Although elevated activity was indicated in some of the experiments, it was not dose-related. At the concentrations tested (0.08 ug/plate, highest dose), 2,4-DNT was not mutagenic in the Salmonella assay before or after ozonation. The highest concentration tested in the Saccharomyces assay, 0.004 percent was not mutagenic or toxic. There was generally a higher number of mitotic recombinants after ozonation, but the response was not dose-related. The products of ozonation of TNT condensate-water mixture (complex nitroaromatics containing primarily 2,4-and 2,6-DNTs) were also tested for mutagenicity. Two new products (m/e 166 and 270) were found in the GC/MS profile. The fragmentation pattern of the m/e 166 compound was found to be consistent with a nitrosonitrotoluene but was not confirmed. Prior to ozonation, the TNT condensate-water mixture was mutagenic in Salmonella assays but not in Saccharomyces. After ozonation, the mixture was weakly mutagenic in only one experiment with TA 1535 and TA 100 in the absence of metabolic activation; thus, activity was considerably reduced after ozonation. A duplicate experiment showed no activity. These mutagenicity results are presented in Table 8.

Carcinogenicity

There are two reports in the literature (NCI, 1978; Lee, et al. 1978) which address the carcinogenicity of 2,4-DNT. A bioassay of practical-grade 2,4-DNT for possible carcinogencity (NCI, 1978)

TABLE 8

Mutagenic Assay Results of Munitions Compounds*

Munitions Compounds	Initial Concentration (ppm)	Reaction Time (min)	Reacted (%)	pH	Salmonella Activity	Saccharomyces Activity	Comments
2,4-Dinitrotoluene	28.3	20	96	8.4/3.8	-/-	-/±	elevated activity in high dose, not dose related
TNT condensate water	35.4	100	9.3	7.2/3.6	±/-	-/-	activity found in one test, reduced by ozonation

*Source: Cotruvo, et al. 1977

was conducted using Fisher 344 rats and $B6C3F_1$ mice. 2,4-DNT was administered in the feed to male and female rats; the low and high time-weighted average doses were 17.6 and 44.0 mg/kg/day for male rats and 25.3 and 63.4 mg/kg/day for female rats, respectively. For male and female mice, the low and high time-weighted average doses were 16.3 and 81.5 mg/kg/day, respectively. Both rats and mice were treated with 2,4-DNT for 78 weeks. In the male rats, a significantly higher incidence of fibroma of the skin and subcutaneous tissue occurred in the high and low dose groups when compared to their respective controls (Table 9). A statistically significant incidence of fibroadenoma of the mammary gland occurred in the treated female rats of the high dose group (Table 10). It should be noted that the above-mentioned tumors were benign.

There were certain unusual neoplasms (i.e., hemangiosarcoma in the subcutis, hemangiosarcoma of the urinary bladder, and prostrate gland adenocarcinoma) that occurred at low incidences in high dose male rats but did not occur in either low dose or control male rats. The authors (NCI, 1978) considered that these tumors were not related to chemical administration.

For the mice, there were no tumors in either sex having a statistically significant positive association between administration of 2,4-DNT and incidence of tumor. As such there is no convincing evidence of tumorigenicity in $B6C3F_1$ mice at the dose levels of 2,4-DNT used in these experiments.

At this point, it is relevant to present some of the comments made regarding this carcinogenesis study by the Data

TABLE 9

Summary of the Significant Primary Tumors at Specific Sites in Male Rats Treated with 2,4-Dinitrotoluene*,[a]

Topography: Morphology	Low Dose Control	High Dose Control	Low Dose	High Dose
Subcutaneous Tissue or Skin: Fibroma[b]	0/46(0.00)	0/25(0.00)	7/49(0.14)	13/49(0.27)
P Values[c]	---	---	P = 0.008	P = 0.003
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.827	2.106
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	96	85

*Source: NCI, 1978

[a]Treated groups received time-weighted average concentrations of 17.6 and 44.0 mg/kg/day in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P<0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

TABLE 10

Summary of the Significant Primary Tumors at Specific Sites in Female Rats Treated with 2,4-Dinitrotoluene*,[a]

Topography: Morphology	Low Dose Control	High Dose Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	9/48(0.19)	4/23(0.17)	12/49(0.24)	23/50(0.46)
P Values[c]	---	---	N.S.	P = 0.016
Relative Risk (Control)[d]	---	---	1.306	2.645
Lower Limit	---	---	0.559	1.062
Upper Limit	---	---	3.183	9.435
Weeks to First Observed Tumor	92	109	83	69

*Source: NCI, 1978

[a]Treated groups received time-weighted average concentrations of 25.3 and 63.4 mg/kg/day in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates lower incidence in the treated group than in the control group.

[d]The 95% confidence interval of the relative risk of the treated group to the control group.

Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens (NCI, 1978):

1. The tumors in the treated rats must be viewed with concern, especially since the maximum tolerated dose may not have been attained.

2. Since 2,4-DNT is an intermediate in the production of dyes, there may be considerable human exposure from its residues in dye products. Hence, there may be a potential for human risk because of the increased tumor incidence seen in the treated rats.

3. The biological activity of 2,4-DNT may be due to its possible conversion to the diamine compound, 2,4-toluenediamine. The rate of its enzymatic conversion may limit its activity.

4. These data do not allow an assessment of human risk.

5. In view of the significant number of benign tumors in the treated rats and widespread human exposure, 2,4-DNT should be considered for retest using another species and route of exposure, especially dermal.

Another bioassay of practical grade 2,4-DNT for possible carcinogenicity was conducted by Lee, et al. (1978) using CD rats (Charles River Breeding Laboratory, Wilmington, Mass.) The high dose, with 2,4-DNT intake of 34.0 mg/kg/day in male rats and 45.0 mg/kg/day in female rats, was quite toxic, causing decreased weight gain and shortened lifespan. Target organs included the blood (toxic anemia), the liver (hepatocellular carcinoma), the testis (aspermatogenesis), and connective tissue in male rats (fibromas), and the mammary tissue in female rats (fibroadenomas). No specific effects were seen on the reproductive process, on chromosomes, or on the metabolism of 2,4-DNT. The middle dose, with 2,4-DNT intake of 3.90 mg/kg/day in male rats and 5.10 mg/kg/day in female rats, was somewhat toxic. It caused similar effects in some, more

susceptible, individual rats. The low dose, with 2,4-DNT intake of 0.57 and 0.71 mg/kg/day in male and female rats respectively, had no apparent toxic effects. The carcinogenicity results for male and female rats are summarized in Tables 11 and 12, respectively.

The interim results (weeks 52 and 55) of a feeding study in rats given 2,4-DNT indicated a significant increase in the incidence of hepatocellular carcinomas in both males and females (Chemical Industry Institute of Toxicology, 1978). Although this study has not yet been published or reviewed in detail, it supports the results of Lee, et al. (1978).

Since 2,4-toluenediamine (2,4-TDA) is a possible metabolic product of 2,4-DNT (as seen in Figure 1) and is mentioned in the critique of the Lee, et al. (1978) study, it is reasonable to discuss briefly the carcinogenicity and mutagenicity of 2,4-TDA.

2,4-TDA is widely used in the production of human hair dyes. Umeda (1955) reported that the repeated subcutaneous injections of 2,4-TDA induced rhabdomyosarcomas in 100 percent of rats treated. Rats fed diets containing 2,4-TDA developed hepatocellular carcinomas (Ito, et al. 1969). Similarly Swiss-Webster mice fed 2,4-TDA showed a high incidence of lung neoplasms (Stoats, 1972). In contrast, the recent study by Giles, et al. (1976) indicates that the 2,4-TDA and other hair dye ingredients did not augment the development of primary lung neoplasms in mice. Skin neoplasms were seen in most groups of Swiss-Webster mice, but the incidence of these tumors in treated animals when compared to control mice, was not significant. The 2,4-TDA under these experimental conditions was found to be nontoxic and noncarcinogenic to the skin of mice.

TABLE 11

Summary of the Male Rats with Tumors
After being Fed 2,4-Dinitrotoluene for 24 months*

Dose (mg/kg/day)	Mammary Tumor/Total	Percent
0	1/37	3
0.57	0/37	0
3.90	0/29	0
34.0	17/23	74

*Source: Lee, et al. 1978

TABLE 12

Separate Tumor Incidence for All Age Groups
Female Rats fed 2,4-DNT*

	Control (0 ppm) (0 mg/kg/day)	0.0015% (15 ppm) (0.71 mg/kg/day)	0.01% (100 ppm) (5.1 mg/kg/day)	0.07% (700 ppm) (45 mg/kg/day)
Liver[a]	0/31	3/43 (N.S.)	3/35 (N.S.)	30/42[d] ($p=3.96 \times 10^{-4}$)
Mammary gland tumor[b]	11/31	12/43 (N.S.)	18/35 (N.S.)	34/43[d] ($p=1.75 \times 10^{-4}$)
Combined mammary gland and liver tumor[c]	11/31	13/43 (N.S.)	18/35 (N.S.)	35/43[d] ($p=7.01 \times 10^{-5}$)

*Source: Lee, et al. 1978

[a]Number of neoplastic nodule or hepatocellular carcinoma animals/no. of anilmals in which livers were examined.

[b]Number of animals with either adenoma, fibroadenoma, fibroma, or adenocarcinoma of the mammary gland/no. of animals in which mammary gland tissues were examined.

[c]The number of animals which had either liver or mamary gland tumors or both/no. of animals in which the liver and mammary glands were examined.

[d]The total number of animals examined microscopically for mammary gland tumors was 43. One animal out of these 43 rats was missing liver tissue, i.e., livers examined were 42. However, the animals which was missing liver tissue had a mammary gland tumor, so it was counted as an animal having a tumor. Therefore, the total number of animals examined 0.07% dose was 43.

On the other hand, it has been shown that 2,4-TDA is a mutagen in several systems. A good correlation between mutagenicity of 2,4-TDA in the Salmonella/ microsome test and morphological transformation in hamster embryo cell system was observed by Shah, et al. (1977). 2,4-TDA usually requires metabolic activation by rat liver microsomal enzymes (S9) for mutagenesis in tester strains TA 1538 and TA 98 (McCann, et al. 1975; Shah, et al. 1977; Dybing, et al. 1977; Pienta, et al. 1977). In contrast, transformation of hamster cells was induced without the addition of external enzymes (Shah, et al. 1977), presumably because the cells can metabolize 2,4-TDA to its active derivatives. There was no mutagenic activity in the strain TA 100, indicating that 2,4-TDA is not a base pair mutagen. The dose-response curves obtained with tester strains TA 1538 and TA 98 demonstrated that 2,4-TDA is metabolized by the S9 activation mixture to a frameshift mutagen (Shah, et al. 1977). 2,4-TDA was also found to be mutagenic in the sex-linked recessive lethal test in Drosophila melanogaster male germ cells (Blijleven, 1977; Fahmy and Fahmy, 1977; Venitt, 1978).

CRITERION FORMULATION

Existing Guidelines and Standards

At present, no standard for exposure to 2,4-DNT in drinking or ambient water has been set in the United States. However, a Russian study (Korolev, et al. 1977) recommends that a maximum permissible concentration in the surface waters should be set at a level of 0.5 mg/l for each DNT isomer.

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value-time weighted average (TLV-TWA) concentration of 1.5 mg of 2,4-DNT per cubic meter of air (1.5 mg/m^3) including dermal exposure for a normal eight-hour workday of a 40-hour workweek (ACGIH, 1978). This value represents the highest level to which nearly all workers may be repeatedly exposed, day afer day, without adverse effect. This TLV-TWA was set by analogy with chemically similar nitro aromatic compounds. A threshold limit value short-term exposure level (TLV-STEL) of 5 mg of 2,4-DNT/m^3 of air was also set by the ACGIH (1978). The TLV-STEL is defined as the maximal allowable concentration to which workers can be exposed for a continuous period of up to 15 minutes without suffering from 1) irritation, 2) chronic or irreversible tissue change, or 3) narcosis of sufficient degree to increase accident proneness, impair self-rescue, or materially reduce work efficiency. No more than four exposures to the TLV-STEL per day are permitted, with at least 60 minutes between exposure periods. Additionally, the daily TLV-TWA must not be exceeded.

Current Levels of Exposure

No data on the extent of human exposure to 2,4-DNT are available in the literature. However, a study of the concentration of explosives in air by isotope dilution analysis (St. John, et al. 1975) reported a concentration of 184 ppb v/v (=1.384 mg/m^3) of 2,4-DNT in air at 25°C, which is very close to the TLV-TWA value noted above.

Special Groups at Risk

The main group expected to be at high risk for exposure to 2,4-DNT is industrial workers involved in the manufacturing or handling of 2,4-DNT in places such as ammunition, dye, and polyurethane plants.

Basis and Derivation of Criteria

Although both bioassays for carcinogenicity were considered in assessing the potential carcinogenic effect of dinitrotoluene (NCI, 1978; Lee, et al. 1978), the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens (NCI, 1978) expressed reservations about the adequacy of its bioassay for use in assessing human risk. Therefore, the criterion was developed from the Lee, et al. (1978) study.

Both of these carcinogencity studies with dietary administration of 2,4-DNT showed increased incidences of fibroadenomas of the subcutaneous tissue and inanition in male rats and fibroadenomas of the mammary gland and inanition in female rats. In addition, the Lee, et al. study showed a significant increase in liver tumors in female rats. It should be noted that both of these bioassays used technical grade 2,4-DNT which contained other DNT isomers as

impurities. The influence of the other isomers and impurities on the carcinogenic activity of technical grade 2,4-DNT cannot be properly assessed at this time.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." 2,4-DNT is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of 2,4-DNT in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of 2,4-DNT corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6}, or 10^{-7} as shown in the following table.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1)		
	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 grams fish and shellfish. (2)	0.011 µg/l	0.11 µg/l	1.1 µg/l
Consumption of fish and shellfish only.	0.91 µg/l	9.1 µg/l	91 µg/l

(1) Calculated by applying a linearized multistage model, as discussed in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document, to the animal bioassay data presented in Appendix II and in Table 12. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000 and so forth.

(2) Approximately 1.2 percent of the 2,4-DNT exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 3.8-fold. The remaining 98.8 percent of 2,4-DNT exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of 2,4-DNT, (1) occurring from the consumption of both drinking water and aquatic life grown in waters containing the corresponding 2,4-DNT concentrations and, (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding 2,4-DNT concentrations. Although total exposure

information for 2,4-DNT is discussed and an estimate of the contributions from other sources of exposure can be made, this data will not be factored into ambient water quality criteria. The criteria presented, therefore, assume an incremental risk from ambient water exposure only.

Results obtained from the linearized multistage model give 1.1 µg/l as the dose level which establishes a carcinogenicity risk level in water for humans of 1 in 100,000. It should be noted that this level is one five-hundredth the level of 0.5 mg/l for surface water recommended in the USSR (Korolev, et al. 1977).

Using the TLV-TWA value for 2,4-DNT of 1.5 mg/m^3 recommended by the ACGIH (1978), the daily occupational exposure gives a value of 5.4 mg of 2,4-DNT per day (see Appendix I for calculation). At an ambient water level of 1.1 µg/l, assuming a daily intake of 2 liters and a daily aquatic organism intake of 6.5 g with a bioaccumulation factor of 3.8, it can be shown (see Appendix I for calculation) that the daily intake of 2,4-DNT is 0.0015 mg/day which is substantially below the occupational exposure level and hence, will not pose a significant additional burden of exposure by those at risk occupationally. This proposed level in ambient water leads to an intake (0.0015 mg/day) which would cause an insignificant effect in terms of contribution to methemoglobinemia (25 mg of 2,4-DNT/l) (Cartwright, 1977; Proctor and Hughes, 1978). It would thus appear that this extrapolation, using female rat data (Lee, et al. 1978) provides a level of ambient water exposure which achieves a high margin of safety.

It should be noted that data are urgently needed in the following areas to evaluate properly any hazard from 2,4-DNT:

1. Monitoring of workers exposed to 2,4-DNT in industries manufacturing or using the chemical.
2. Monitoring of public water supplies and industrial and municipal effluents to determine an expected range of concentrations under differing environmental conditions.
3. More detailed studies on the pharmacokinetics of 2,4-DNT using several animal species and if possible, occupationally exposed humans.
4. Evaluation of chronic toxicity and teratogenicity using currently acceptable techniques.
5. Detailed and definitive mutagenicity studies of 2,4-DNT and its metabolites using several assay systems such as: a) Salmonella/microsomal, b) dominant lethal, c) Drosophila, and d) host mediated assay.
6. More definitive studies on the carcinogenicity of 2,4-DNT and its metabolites using several animal species (and if possible, occupationally exposed humans) using oral and dermal routes.

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APPENDIX I

1. Calculation of Daily Occupational Exposure level of 2,4-Dinitrotoluene based on its Threshold Limit Value-Time Weighted Average (TLV-TWA) concentration (ACGIH, 1978):

TLV-TWA for 2,4-DNT = 1.5 mg/m^3 of air for a normal 8-hour workday or 40-hour workweek

$$= 1.5 \times 10^{-3} \frac{mg}{\text{liter of air}}$$

$$= 1.5 \frac{\mu g}{\text{liter of air}}$$

Therefore, the daily occupational level for

$$2,4\text{-DNT} = \frac{1.5\ \mu g}{\text{liter}} \times \frac{7.5\ \text{liter of air}}{\text{minute}} \times \frac{60\ \text{minute}}{\text{hour}} \times \frac{8\ \text{hour}}{\text{day}}$$

$$= 5,400\ \mu g$$

$$= 5.4\ mg$$

where 7.5 liter of air is the ventilation rate for an average 70 kg man doing moderately hard work (Kamon, 1979).

2. Calculation of Daily Intake Level of 2,4-DNT:

The assumptions used for this calculation are:

a) Bioaccumulation factor of 3.8 as determined for the bluegill sunfish (U.S. EPA report, Duluth, Minnesota),

b) Average weight of aquatic organisms consumed per day is 6.5 g, and

c) Consumption of water per person per day is 2 liters over a period of 70 years.

d) A concentration of 2,4-DNT in water of 740 ng/l.

The concentration of 2,4-DNT in fish = 740 x 3.8 x 0.0065 = 18 ng from aquatic organisms

Daily intake of 2,4-DNT from 2 liters of drinking water = 740 ng/l x 2 = 1,480 ng

Total intake/day = 1,480 + 18 ng or 1,498 ng (1.50 µg or .00150 mg)

APPENDIX II

Summary and Conclusions Regarding the Carcinogenicity of 2,4-Dinitrotoluene*

2,4-Dinitrotoluene (2,4-DNT) is a pale yellow crystalline solid with a melting point of 70°C and has a moderate fire explosion risk. A combined U.S. production of approximately 272 billion pounds of 2,4- and 2,6-dinitrotoluene isomers was reported in 1975. 2,4-DNT is widely used as a raw material for dyestuffs and for urethane polymers, as a modifier for smokeless powders, and as a gelatinizing and waterproofing agent in military and commercial explosives.

The reports concerning the mutagenicity of 2,4-DNT are limited and their results conflicting. However, this compound was found to be mutagenic in the dominant lethal assay in rats and in microbial tests using Salmonella typhimurium TA1535 indicating base-pair substitution.

Two reports concerning the carcinogenicity of 2,4-DNT are in the literature. The first is a National Cancer Institute (NCI) two-year bioassay in male and female Fisher 344 rats and $B6C3F_1$ mice fed 2,4-DNT (1978). The major pathologic findings were present in the rats. These included fibromas of the skin and subcutaneous tissues in males and fibroadenomas of the mammary gland in the females. These tumors are benign and were dose-related. The mice had no statistically significant carcinogenic response to the administration of 2,4-dinitrotoluene.

The second study relating oral administration of 2,4-DNT to carcinogenicity was a bioassay in male and female Charles River CD rats and CD-1 mice fed 2,4-DNT for two years (Lee, et al. 1978).

The major pathologic findings in the rats included a significant increase of hepatocellular carcinomas ($p = 7.1 \times 10^{-6}$) and neoplastic nodules ($p = 0.01$) in the liver of females, mammary gland tumors of the female ($p = 8.3 \times 10^{-5}$) and the suspicious increase of hepatocellular carcinomas of the liver in males. All of these rat tumors were in high dose animals. The pathologic finding in the mice was the highly significant ($p = 1.5 \times 10^{-7}$) increase of kidney tumors in the males of the middle dose group.

The induction of hepatocellular carcinomas, hepatocellular neoplastic nodules and mammary tumors in female rats and kidney tumors in male mice from the administration of 2,4-dinitrotoluene indicates that it is likely to be a human carcinogen.

The water quality criterion for 2,4-dinitrotoluene is based on the induction of mammary tumors, hepatocellular carcinomas, and hepatocellular neoplastic nodules in female Charles River CD rats fed various doses of 2,4-DNT for 24 months (Lee, et al. 1978). It is concluded that the water concentration of 2,4-dinitrotoluene should be less than 1.1 µg/l in order to keep the lifetime cancer risk below 10^{-5}.

*This summary has been prepared and approved by the Carcinogens Assessment Group of EPA on June 19, 1979.

Summary of Pertinent Data

The water quality criterion for 2,4-dinitrotoluene is derived from the oncogenic effects observed in the mammary gland and liver of female Charles River CD rats fed various doses of 2,4-DNT for 24 months, with the surviving animals sacrificed one month later. The incidence of mammary and/or liver tumors is listed below for the various doses, along with other parameters used in the extrapolation:

Dose (mg/kg/day)	Incidence (no. responding/no. tested)
0.0	11/31
0.75	13/43
5.0	18/35
35.0	35/43

le = 720 days

Le = 750 days

L = 750 days

w = 0.464 kg

R = 3.8 l/kg

With these paramethers the carcinogenic potency factor for humans, q_1^*, is 3.6965×10^{-2} $(mg/kg/day)^{-1}$. The resulting water concentration of 2,4-dinitrotoluene calculated to keep the individual lifetime cancer risk below 10^{-5} is 1.1 µg/l.