DINITROTOLUENE

Ambient Water Quality Criteria

Criteria and Standards Division
Office of Water Planning and Standards
U.S. Environmental Protection Agency
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CRITERION DOCUMENT

DINITROTOLUENES

CRITERIA

Aquatic Life

2,3-dinitrotoluene

For 2,3-dinitrotoluene the criterion to protect freshwater aquatic life as derived using the Guidelines is 12 µg/l as a 24-hour average and the concentration should not exceed 27 µg/l at any time.

For 2,3-dinitrotoluene the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 4.4 µg/l as a 24-hour average and the concentration should not exceed 10 µg/l at any time.

2,4-dinitrotoluene

For 2,4-dinitrotoluene the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 620 µg/l as a 24-hour average and the concentration should not exceed 1,400 µg/l at any time.

For saltwater aquatic life, no criterion for 2,4-dinitrotoluene can be derived using the Guidelines, and there are insufficient data to estimate a criterion using other procedures.

Human Health

For the maximum protection of human health from the potential carcinogenic effects of exposure to 2,4-dinitrotoluene through ingestion of water and contaminated aquatic organisms, the ambient water concentration is zero. Concentrations of 2,4-dinitrotoluene estimated to result in additional lifetime cancer risks ranging from no additional risk to an additional risk of 1 in 100,000 are

presented in the Criterion Formulation section of this document. The Agency is considering setting criteria at an interim target risk level in the range of 10^{-5}, 10^{-6}, or 10^{-7} with corresponding criteria of 740 ng/l, 74.0 ng/l, and 7.4 ng/l, respectively.

DINITROTOLUENES

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 has been shown to enter the body through inhalation of vapors or dust particles, ingestion of contaminated food, and absorption through the skin. As a result of exposure to DNT, workers have experienced muscular weakness, headaches, and dizziness; this exposure also has been suspected of causing pallor, cyanosis, and anemia.

DNT is produced by nitration of toluene to nitrotoluene to dinitrotoluene in a nitric and sulfuric acid solution (Lopez, 1977). In 1975, the production of 2,4- and 2,6-DNT in the United States was 264,030 metric tons (U.S. Int. Trade Comm., 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).

2,4-DNT has a molecular weight of 182.14, a melting point of 71°C, a boiling point of 300°C with decomposition, and a density of 1.3208 at 71°C (Weast, 1975). Its solubility in water is 270 mg/l water at 22°C; 94 g/l ether at 22°C and 21.9 g/l carbon disulfide at 17°C (Kirk and Othmer, 1967). It is also readily soluble in ethanol at 15°C (30.5 g/l) (Kirk and Othmer, 1967).

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).

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). Mixtures of dinitrotoluene isomers are intermediates in the manufacture of toluene diisocyanates (Wiseman, 1972). Because of the deactivating effect of the two nitro groups in dinitrotoluenes, the synthesis of trinitrotoluene (TNT) does not occur as readily (Wiseman, 1972).

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AQUATIC LIFE TOXICOLOGY*

FRESHWATER ORGANISMS

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

The unadjusted 96-hour LC50 for the fathead minnow and 2,4-dinitrotoluene is 31,000 µg/l (Table 1). After adjustment for test methods and species sensitivity, the Final Fish Acute Value for this compound is 4,300 µg/l. The unadjusted 96-hour LC50 for the more toxic 2,3-dinitrotoluene and the bluegill is 330 µg/l, and this datum results in a Final Fish Acute Value of 46 µg/l (Table 1).

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life [43 FR 21506 (May 18, 1978) and 43 FR 29028 (July 5, 1978)] 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 the calculations for deriving various measures of toxicity as described in the Guidelines.

Forty-eight-hour EC50 values are available for Daphnia magna for both 2,3- and 2,4-dinitrotoluene and are 660 and 35,000 µg/l, respectively (Table 2). The Final Invertebrate Acute Values are 27 and 1,400 µg/l for 2,3- and 2,4-dinitrotoluene, respectively, and since these concentrations are lower than the comparable concentrations for fish, they also become the Final Acute Values.

Chronic Toxicity

The chronic value for 2,3-dinitrotoluene, derived from a embryo-larval test with the fathead minnow, is 116 µg/l (Table 3) and is based on survival of these life stages (U.S. EPA, 1978). The Final Fish Chronic Value is 17 µg/l, and this concentration also becomes the Final Chronic Value for 2,3-dinitrotoluene in the absence of data on any invertebrate species.

Plant Effects

Cell numbers of the alga, Selenastrum capricornutum, were reduced by 50 percent at a concentration of 2,3-dinitrotoluene of 1,370 µg/l (Table 4). A comparable inhibition in chlorophyll a occurred at a concentration of 1,620 µg/l. No other data on dinitrotoluenes and freshwater plants are available.

Residues

No measured steady-state bioconcentration factor (BCF) is available for 2,4-dinitrotoluene. A BCF can be estimated using the octanol-water partition coefficient of 100. This coefficient is used to derive an estimated BCF of 19 for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the consuming species of concern contains a significantly different lipid content, an appropriate adjustment in the estimated BCF should be made.

CRITERION FORMULATION

Freshwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

2,3-dinitrotoluene

Final Fish Acute Value = 46 µg/l

Final Invertebrate Acute Value = 27 µg/l

Final Acute Value = 27 µg/l

Final Fish Chronic Value = 17 µg/l

Final Invertebrate Chronic Value = not available

Final Plant Value = 1,400 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 17 µg/l

0.44 x Final Acute Value = 12 µg/l

2,4-dinitrotoluene

Final Fish Acute Value = 4,300 µg/l

Final Invertebrate Acute Value = 1,400 µg/l

Final Acute Value = 1,400 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = not available

Residue Limited Toxicant Concentration = not available

Final Chronic Value = not available

0.44 x Final Acute Value = 620 µg/l

2,3-dinitrotoluene

The maximum concentration of 2,3-dinitrotoluene is the Final Acute Value of 27 µg/l and the 24-hour average concentration is

0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,3-dinitrotoluene the criterion to protect freshwater aquatic life as derived using the Guidelines is 12 µg/l as a 24-hour average and the concentration should not exceed 27 µg/l at any time.

2,4-dinitrotoluene

No freshwater criterion can be derived for 2,4-dinitrotoluene using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available.

Results obtained with 2,3-dinitrotoluene and freshwater organisms indicate how a criterion may be estimated for 2,4-dinitrotoluene and freshwater organisms.

For 2,3-dinitrotoluene and freshwater organisms 0.44 times the Final Acute Value is less than the Final Chronic Value based on an embryo-larval test with the fathead minnow. Therefore, a reasonable estimate of a criterion for 2,4-dinitrotoluene and freshwater organisms would be 0.44 times the Final Acute Value.

The maximum concentration of 2,4-dinitrotoluene is the Final Acute Value of 1,400 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,4-dinitrotoluene the criterion to protect freshwater aquatic life as derived using procedures other than the Guidelines is 620 µg/l as a 24-hour average and the concentration should not exceed 1,400 µg/l at any time.

Table 1. Freshwater fish acute values for dinitrotoluenes

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)	Reference
Fathead minnow, Pimephales promelas	S	U	2,4-dinitro-toluene	96	31,000	17,000	U.S. Army, 1976
Bluegill, Lepomis macrochirus	S	U	2,3-dinitro-toluene	96	330	180	U.S. EPA, 1978

* S = static

** U = unmeasured

Geometric mean of adjusted values: 2,3-dinitrotoluene = 180 µg/l $\frac{180}{3.9}$ = 46 µg/l

2,4-dinitrotoluene = 17,000 µg/l $\frac{17,000}{3.9}$ = 4,300 µg/l

Table 2. Freshwater invertebrate acute values for dinitrotoluenes

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)	Reference
Cladoceran, Daphnia magna	S	U	2,3-dinitro-toluene	48	660	560	U.S. EPA, 1978
Cladoceran, Daphnia magna	S	U	2,4-dinitro-toluene	48	35,000	30,000	U.S. Army, 1976

* S = static

** U = unmeasured

Geometric mean of adjusted values: 2,3-dinitrotoluene = 560 µg/l $\frac{560}{21}$ = 27 µg/l

2,4-dinitrotoluene = 30,000 µg/l $\frac{30,000}{21}$ = 1,400 µg/l

Table 3. Freshwater fish chronic values for dinitrotoluenes (U.S. EPA, 1978)

Organism	Test*	Limits (ug/l)	Chronic Value (ug/l)
		2,3-dinitrotoluene	
Fathead minnow, Pimephales promelas	E-L	200-270	116

* E-L = embryo-larval

Geometric mean of chronic values: 2,3-dinitrotoluene = 116 µg/l $\frac{116}{6.7}$ = 17 µg/l

Lowest chronic value = 116 µg/l

Table 4. Freshwater plant effects for dinitrotoluenes (U.S. EPA, 1978)

Organism	Effect	Concentration (ug/l)
	2,3-dinitrotoluene	
Alga, Selenastrum capricornutum	EC50 96-hr cell numbers	1,370
Alga, Selenastrum capricornutum	EC50 96-hr chlorophyll a	1,620

Lowest plant value: 2,3-dinitrotoluene = 1,370 µg/l

SALTWATER ORGANISMS

Introduction

Acute toxicity tests using static conditions have been conducted with 2,3-dinitrotoluene and the sheepshead minnow, the mysid shrimp Mysidopsis bahia, and an alga, Skeletonema costatum. The LC50 and EC50 values range from 370 µg/l for algal cell numbers to 2,280 µg/l for the sheepshead minnow. No other data on any dinitrotoluene are available.

Acute Toxicity

The Final Fish Acute Value for 2,3-dinitrotoluene is 340 µg/l (Table 5) and is based on a single 96-hour static test with the sheepshead minnow (U.S. EPA, 1978).

The unadjusted 96-hour LC50 for 2,3-dinitrotoluene and Mysidopsis bahia, is 590 µg/l (Table 6) and after adjustment for test methods and species sensitivity, a Final Invertebrate Acute Value of 10 µg/l is obtained. This also becomes the Final Acute Value for 2,3-dinitrotoluene and saltwater organisms since the comparable acute value for fish is higher.

Chronic Toxicity

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

Plant Effects

A 50 percent reduction in cell numbers of the alga, Skeletonema costatum, occurred at a concentration of 370 µg

2,3-dinitrotoluene/l (Table 7). There was a 50 percent inhibition of chlorophyll a production at 400 µg/l.

Residues

No measured steady-state bioconcentration factor (BCF) is available for 2,4-dinitrotoluene. A BCF can be estimated using the octanol-water partition coefficient of 100. This coefficient is used to derive an estimated BCF of 19 for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the consuming species of concern contains a significantly different lipid content, an appropriate adjustment in the estimated BCF should be made.

CRITERION FORMULATION

Saltwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

2,3-dinitrotoluene

Final Fish Acute Value = 340 µg/l

Final Invertebrate Acute Value = 10 µg/l

Final Acute Value = 10 µg/l

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 370 µg/l

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 370 µg/l d for any dinitrotoluene

0.44 x Final Acute Value = 4.4 µg/l Value for either

No saltwater criterion can be derived for any dinitrotoluene using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available.

Results obtained with 2,3-dinitrotoluene and freshwater organisms indicate how a criterion may be estimated for 2,4-dinitrotoluene and saltwater organisms.

For 2,3-dinitrotoluene and freshwater organisms 0.44 times the Final Acute Value is less than the Final Chronic Value based on an embryo-larval test with the fathead minnow. Therefore, a reasonable estimate of a criterion for 2,3-dinitrotoluene and saltwater organisms would be 0.44 times the Final Acute Value.

The maximum concentration of 2,3-dinitrotoluene is the Final Acute Value of 10 µg/l and the estimated 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on saltwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For 2,3-dinitrotoluene the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 4.4 µg/l as a 24-hour average and the concentration should not exceed 10 µg/l at any time.

Table 5. Marine fish acute values for dinitrotoluenes (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)
Sheepshead minnow, Cyprinodon variegatus	S	U	2,3-dinitrotoluene	96	2,280	1,246

* S = static

** U = unmeasured

Geometric mean of adjusted values for 2,3-dinitrotoluene = 1,246 µg/l $\frac{1,246}{3.7}$ = 340 µg/l

Table 6. Marine invertebrate acute values for dinitrotoluenes (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc.**	Chemical Description	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Mysid shrimp, Mysidopsis bahia	S	U	2,3-dinitrotoluene	96	590	500

* S = static

** U = unmeasured

Geometric mean of adjusted values for 2,3-dinitrotoluene = 500 µg/l $\frac{500}{49}$ = 10 µg/l

Table 7. Marine plant effects for dinitrotoluenes (U.S. EPA, 1978)

Organism	Effect	Concentration (ug/l)
		2,3-Dinitrotoluene
Alga, *Skeletonema costatum*	EC50 96-hr chlorophyll a	400
Alga, *Skeletonema costatum*	EC50 96-hr cell numbers	370

Lowest plant value: 2,3-dinitrotoluene = 370 µg/l

* S = static

** U = unmeasured

Geometric mean of adjusted values for 2,3-dinitrotoluene = 1,246 µg/l $\frac{1,246}{3.7}$ = 340 µg

REFERENCES

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2,4-DINITROTOLUENE

Mammalian Toxicology and Human Health Effects

EXPOSURE

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. This process produces a 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 (1977) 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,

TABLE 1

Some Physical Constants of 2,4-Dinitrotoluene
(Data collected from Kirk and Othmer, 1967;
St. John, et al. 1975; Weast, 1978)

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

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 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 is 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 (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 HPLC (Walsh, et al. 1973; Doali and Juhasz, 1974, Stanford, 1977; Natl. Inst. Occup. Safety Health Manual of Analytical Methods, 1978), and spec-

troscopic methods such as infrared (Priestera, et al. 1960) or ultraviolet (Conduit, 1959) spectrophotometry, 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).

Ingestion from Water

2,4-DNT has limited solubility (270 mg/liter at 22°C) in water as noted in Table 1. Possible sources of 2,4-DNT in the aqueous environment-either surface water, ground water or drinking water-are from the dumping of chemical wastes and from accidental loss during transfer and transport.

Dinitrotoluene waste products are dumped into surface water or sewage by manufacturing industries that make dyes, isocyanates, polyurethans, and munitions. The occurrence of organic micropollutants due to the dumping of aromatic nitro and amino compounds in the river water has been reported by Meijers and Van der Leer (1976). The pollution of the rivers Rhine and Maas in the Netherlands by these aromatics and oils was examined by extracting water samples in hexane followed by analyzing the extracts by gas chromatograph/mass spectrometry (GC/MS). The results showed that the river Rhine is heavily polluted by oil, a number of aromatic hydrocarbons, aromatic amines and aromatic nitro compounds including 2,4-DNT. The river Maas, 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 accidently spilled during the process of transfer and/or transportation. No specific incidences of this type have 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 investi-

gators (Schott, et al. 1943; Ruchhoft, et al. 1945; Ruchhoft and Norris, 1946; Rogovskaya, 1951; Nason, 1956; Anon, 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 on 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/liter 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 water to the concentration in aquatic organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the nineteen major species identified in the survey and

data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

Group	Consumption (Percent)	Weighted Average Percent Lipids
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

No measured steady-state bioconcentration factor (BCF) is available for 2,4-dinitrotoluene, but the equation "Log BCF = 0.76 Log P - 0.23" can be used (Veith, et al. Manuscript) to estimate the BCF for aquatic organisms that contain about eight percent lipids from the octanol-water partition coefficient (P). Based on an octanol-water partition coefficient of 100, the steady-state bioconcentration factor for 2,4-dinitrotoluene is estimated to be 19. An adjustment factor of 2.3/8.0 = 0.2875 can be used to adjust the estimated BCF from the 8.0 percent lipids on which the equation is based to the 2.3 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 19 x 0.2875 = 5.5.

Inhalation

The current estimate in the United States for the number of individuals involved in the manufacture of 2,4-DNT is not available at present. But the U.S. International Trade Commission (1977) 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 population may be at risk.

Inhalation has been reported to be one of the major routes of exposure of 2,4-DNT either in its particulate or vapor state. The effects from inhalation exposure to 2,4-DNT are caused by 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., as noted in Table 1, 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 (Threshold Limit Values,

1978). This TLV was set by analogy with chemically similar nitro aromatic compounds (American Conference of Governmental Industrial Hygienists, 1974).

Because of the availability of only limited 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 absorption 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 ratio of radioactivity in 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.

Another study examining the excretion and distribution of tritium-labeled 2,4-dinitrotoluene (^{3}H-2,4-DNT) in the rat has been reported recently (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 1.1 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 was found in only trace quantities. In all, about 46 percent of the radioactivity administered was excreted in the feces and urine during the 7 days (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 by 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
(From Mori, et al. 1977)

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.

TABLE 3

Remaining Radioactivity in the Tissues of Rat
Seven Days after Administration of 3H-2, 4-DNT
(From Mori, et al. 1977)

Tissue	Radioactivity		
	dpm per 100_3mg 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

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 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 (by 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-nitro-toluene; 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.

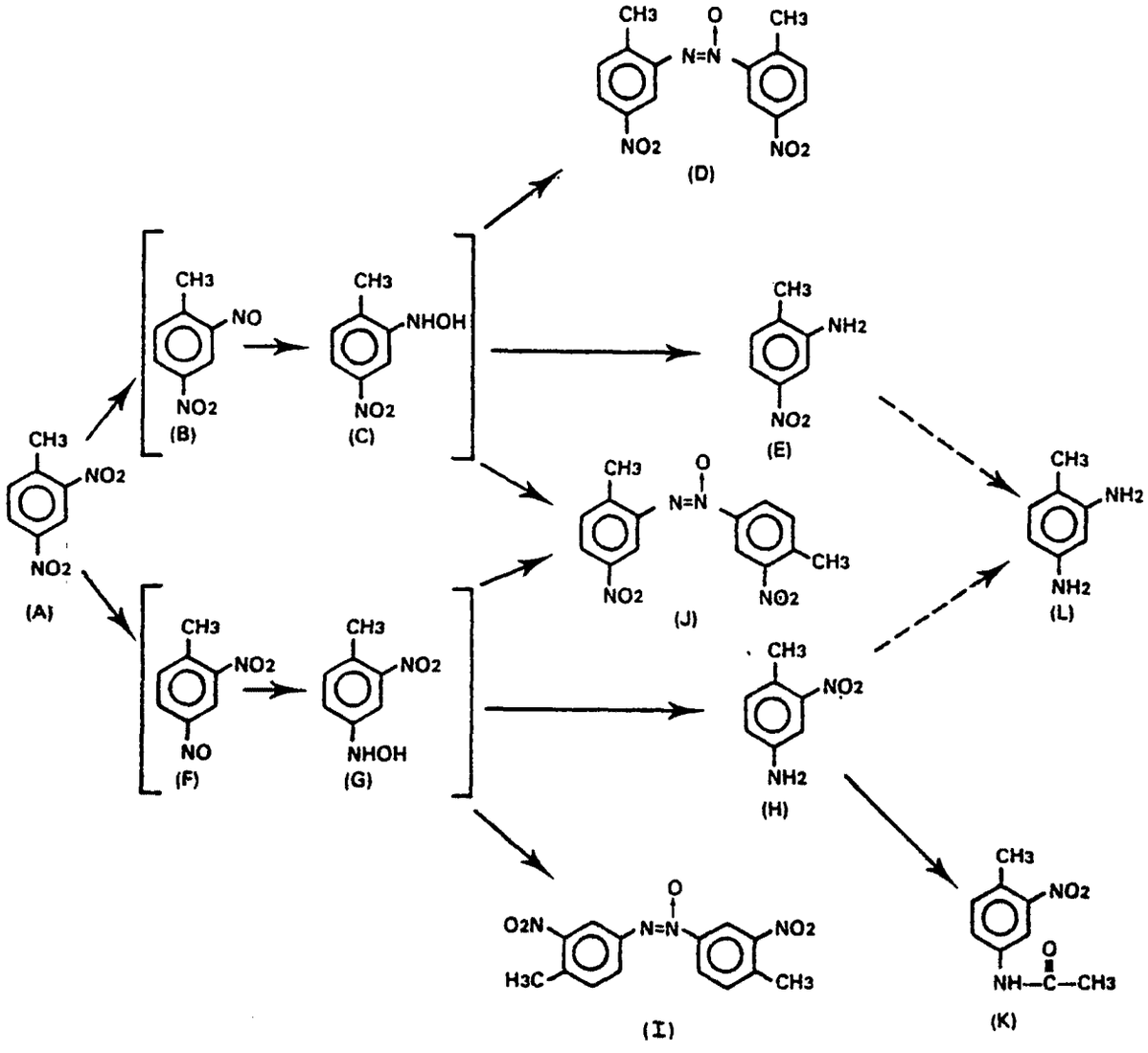

FIGURE 1.

Proposed Pathways for the formation of Biotransformation Products from 2,4-Dinitrotoluene (A)
(Taken from McCormick, et al. 1978)
The hypothetical nitroso and hydroxylamino intermediates are enclosed 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

In a study of the microbial transformation of 2,4-DNT, 2,4,6-TNT and other nitroaromatic compounds by anaerobic bacterial 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; Dobbin 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, from an analogy of metabolism of 2,4,6-TNT with 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, Sub-acute, 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 bring about a chemical change of the blood oxyhemoglobin into methemoglobin (basically, 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

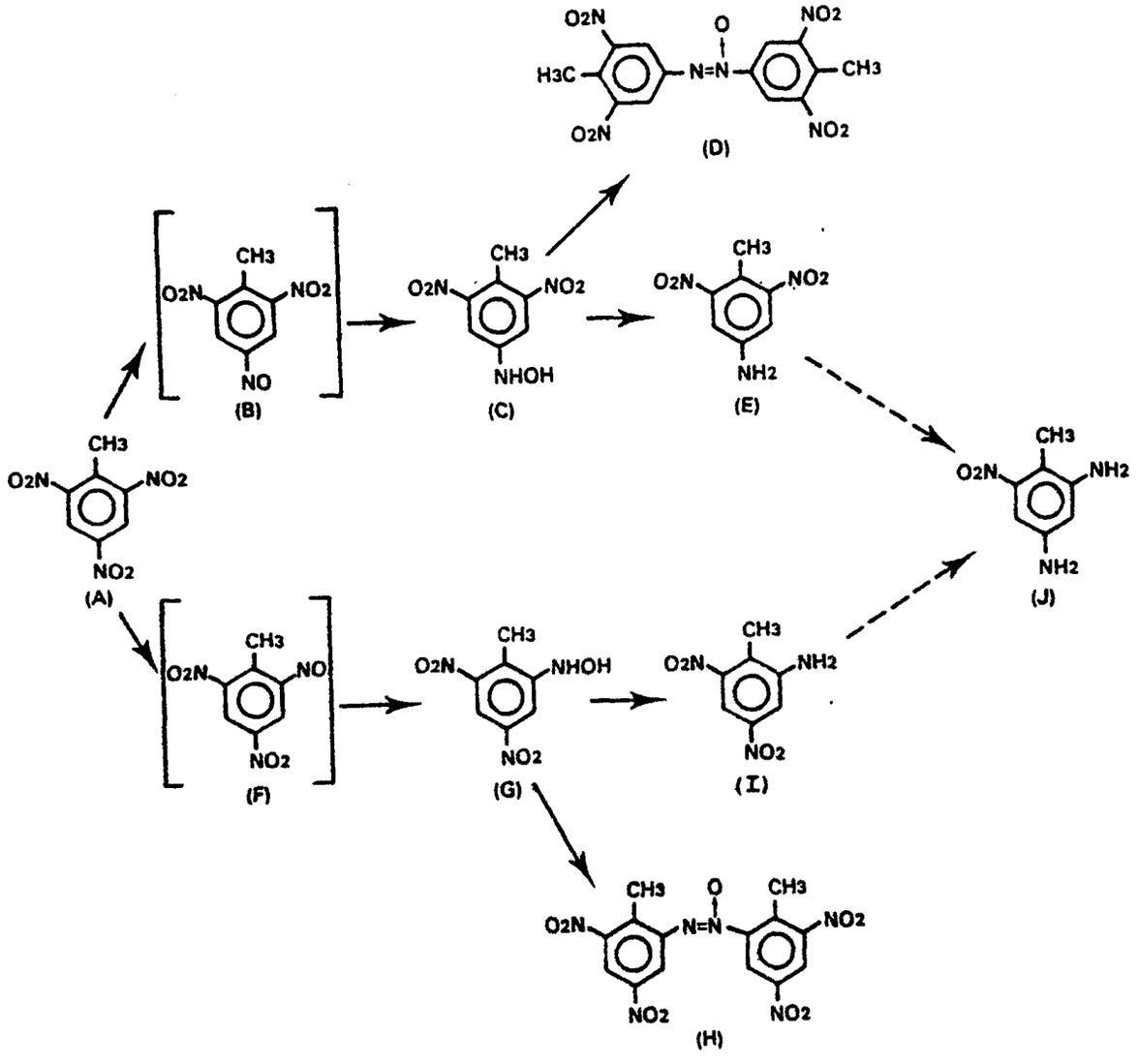

FIGURE 2

Proposed Pathways for the formation of Biotransformation Products from 2,4,6-Trinitrotoluene (A) (Taken from Williams, 1959; Won, et al. 1974). 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

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 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 such compounds as nitrobenzenes, nitrotoluenes and phenylhydrazines occasionally exhibit Heinz bodies in their blood (Hughes and Treon, 1954; De Bruin, 1976).

Inactivation of the hemoglobin under the effect of 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 LD50 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 LD50 values to rats for one to three months, the hemotoxicity of the nitrotoluenes decreased in the order: trinitrotoluene > dinitrotoluene > m-nitrotoluene, p-nitrotoluene > 0-nitrotoluene.

Cyanosis due to the absorption of 2,4-DNT occurs when the methemoglobin concentration 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). Because of the increased vapor pressure with higher ambient temperatures, there is, in general, an increased susceptibility to cyanosis from exposure to 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 in cod liver oil, until a total of 24 ml has been given, without any toxic effect. Similarly Zieger (1913) observed no toxic effects from the inhalation of vapors, whereas in the experience of Kuhls (1908), the subcutaneous injection in cats of 0.05 to 0.5 g of 2,4-DNT dissolved in mineral oil resulted in death after periods of 2 to 23 days. Regarding the possibility of absorption

through the skin, Dambleff (1908) found in rabbits no indication of a toxic action by this route, and similarly Kuhls (1908) observed in cats no toxic effects from the cutaneous administration of 0.3 g/kg body weight, while Zieger (1913) found two doses of 5 g each were fatal to cats in eight hours.

A list of the toxic doses for a number of animal species is presented in Table 4. The rat oral LD50 values listed in Table 4 are comparable to those of nitrobenzene and 2,6-DNT. The mouse 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.

TABLE 4

Acute Toxic Levels of 2,4-Dinitrotoluene for Different Species
(Data collected from Spector, 1956; Fairchild, et al. 1977; Vernot, et al. 1977)

Species	Route	Toxicity	Dose (mg/kg)
Rat	Oral	LD50	268
Mouse	Oral	LD50	1625
Cat	Oral	MLD	27
	S.C.	LDLo	50-500

S.C. - subcutaneous; LDLo - lowest published lethal dose; LD50 - lethal dose 50 percent kill; MLD - minimum lethal dose

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, oppression 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 slow and sluggish 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 known active 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,

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

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 <u>in vivo</u> metabolism;

TABLE 5

Symptoms Presented by 154 2,4-Dinitrotoluene Workers
(From McGee, et al. 1942)

Symptom	Screening House Number of Workmen	Coating House and Air dry Number of Workmen	Total Number	Total 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

TABLE 6

Clinical Findings in 154 2,4-Dinitrotoluene Workers
(From McGee, et al. 1942)

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

he believes 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 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

TABLE 7

Methemoglobin-forming Capacity of Some Aromatic Nitro and Amino Compounds in Cat
(Data collected from Hamblin, 1963; De Bruin, 1976)

Compound	Molecular ratio*
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

*Molar ratio of methemoglobin formed to dose of test compound

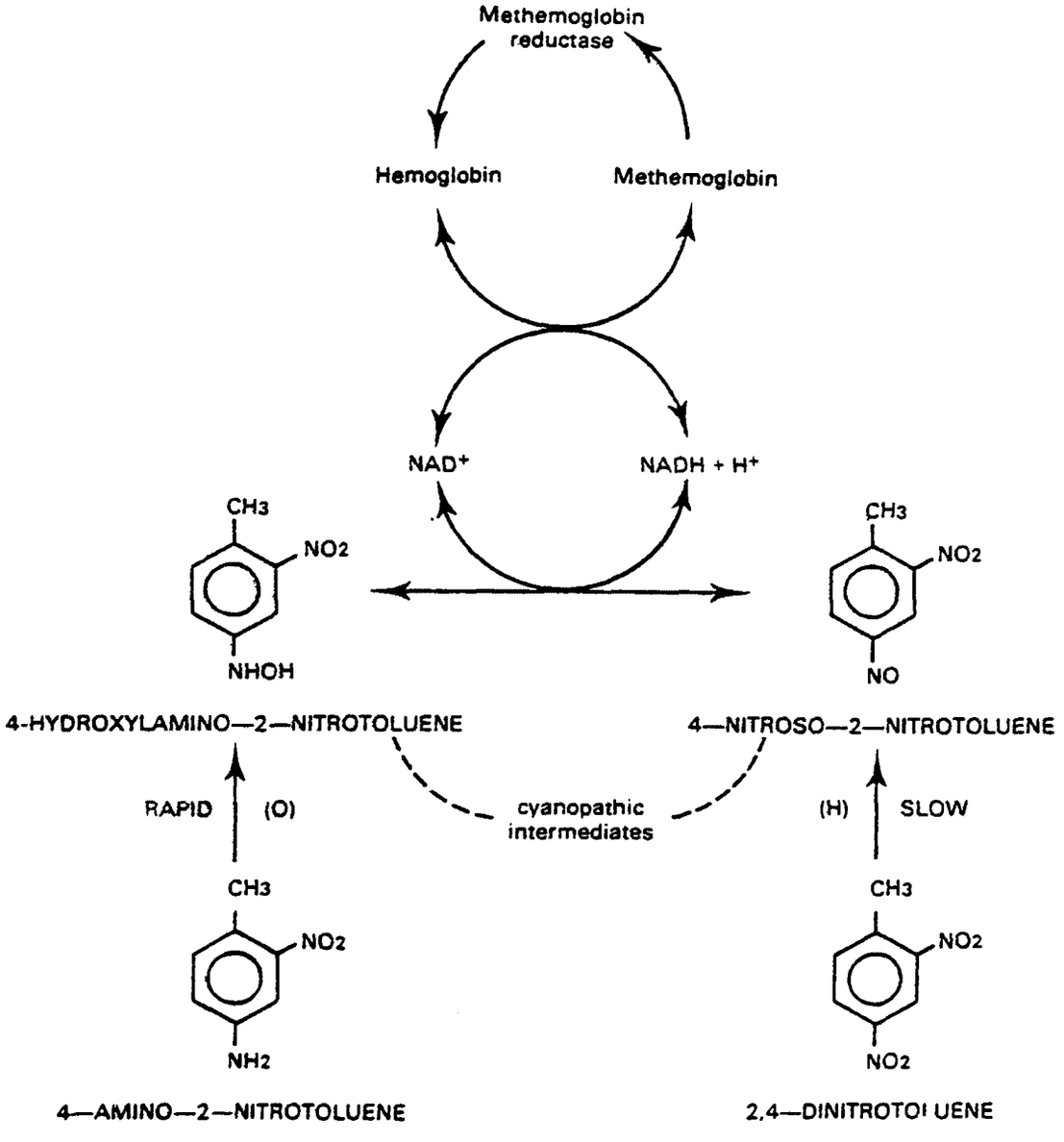

FIGURE 3

Suggested Metabolic Pathway for Cyanosis by 2,4-Dinitrotoluene based upon data from related compounds.

no toxic effects. All species showed methemoglobinemia and anemia with reticulocytosis. Characteristic tissue lesions were extramedullary hematopoeisis in the spleen and liver, gliosis and demy-elination 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 of 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) also studied 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-K1 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.

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 such 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. 1973) 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 these 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 µg/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 (a mixture of complex nitroaromatics containing primarily 2,4-and 2,6-DNT's) were also tested for mutagenicity. Two new products (m/e 166 and 270) were found in the GC/MC 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 afer ozonation. A duplicate experiment showed no

activity. These mutagenicity results are presented in Table 8.

Carcinogencity

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) was conducted using Fisher 344 rats and B6C3F1 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.

TABLE 8

Mutagenic Assay Results
of Munitions Compounds
(From Cotruvo, et al. 1977)

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

TABLE 9

Summary of the Significant Primary Tumors at
Specific Sites in Male Rats Treated with 2,4-Dinitrotoluene[a]
(From NCI, 1978)

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

[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.

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 B6C3F1 mice at the dose levels of 2,4-DNT used in these experiments. The possibility of a negative association between administration and incidence was observed for pituitary adenomas in female mice and for alveolar/bronchiolar neoplasms in male mice.

At this point, it is relevant to present some of the comments made regarding this carcinogenesis study by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Envirnomental 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.

TABLE 10

Summary of the Significant Primary Tumors at
Specific Sites in Female Rats Treated with 2,4-Dinitrotoluene[a]
(From NCI, 1978)

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

[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.

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 life span. 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.

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 above 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

TABLE 11

Summary of the Male Rats with Apparent Tumors
After being Fed 2,4-Dinitrotoluene for 18 months
(From Lee, et al. 1978)

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

TABLE 12

Summary of the Female Rats with Apparent Tumors
After being Fed 2,4-Dinitrotoluene for 18 months
(From Lee, et al. 1978)

Dose (mg/kg/day	Tumor/Total	Percent
0	8/29	28
0.71	11/40	28
5.10	10/27	37
45.0	28/32	88

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.

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 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 Drosophilia 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 (Am. Conf. Gov. Ind. Hyg.) 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 40-hour workweek (Am. Conf. Gov. Ind. Hyg. 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 (Am. Conf. Gov. Ind. Hyg. 1978). The TLV-STEL is defined as the maximal allowable concentration to which workers can be exposed for a period of up to 15 minutes continuously 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, and the daily TLV-TWA must also 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 Criterion

The data from the bioassay of 2,4-DNT for possible carcinogenicity obtained by the National Cancer Institute (1978) and Lee, et al. (1978) were used for the determination of a water quality criterion for the protection of human health. It should be noted at this point, however, that the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens (NCI, 1978) expressed reservations about the adequacy of this bioassay for use in assessing human risk. Nevertheless, the criterion was developed from the animal carcinogenicity data from these two studies by utilizing the linear non-threshold model (see Appendix I). The rat 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.

Under the Consent Decree in NRDC vs 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 table below.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1)			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 18.7 grams fish and shellfish. (2)		7.4 ng/l	74.0 ng/l	740 ng/l
Consumption of fish and shellfish only.		.156 µg/l	1.56 µg/l	15.6 µg/l

(1) Calculated by applying a modified "one-hit" extrapolation model described in the FR 15926, 1979 to the animal bioassay data presented in Appendix I and in Table 9. 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, 1000 and so forth.

(2) Approximately five percent of the DNT exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 5.5 fold. The remaining 95 percent of DNT exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of DNT, (1) occurring from the consumption of both drinking water and aquatic life grown in waters containing the corresponding DNT concentrations and, (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding DNT concentrations. Although total exposure information for chloroform 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 linear non-threshold model give a value of 740 ng/liter as the lowest value obtained, the dose level which establishes a carcinogenicity risk level in water for humans of 1 in 100,000. This was obtained from the study of fibroadenomas of the mammary gland and inanition of the female rats (Lee, et al., 1978). It should be noted that this level is 1/500 the level of 0.5 mg/liter for surface water recommended in the U.S.S.R. (Korolev, et al. 1977).

Using the TLV-TWA value of 1.5 mg/m^3 of air for 2,4-DNT recommended by the Am. Conf. Gov. Ind. Hyg. (1978), the daily occupational exposure gives a value of 5.4 mg of 2,4-DNT per day (see Appendix II for calculation). At an ambient water level of 740 ng/liter, assuming a daily intake of 2 liters and a daily aquatic organism intake of 18.7 g with a bioaccumulation factor of 5.5, it can be shown (see Appendix II for calculation) that the daily intake of 2,4-DNT is 0.0016 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 (0.0016 mg/day) which would cause an insignificant effect in terms of contribution to methemoglobinemia (25 mg of 2,4-DNT/liter) (Cartwright, 1977; Proctor and Hughes, 1978). It would thus appear that the linear non-threshold model, using female rat data on fibroadenomas of the mammary gland and inanition (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

Carcinogenicity Risk Assessment by Extrapolation from Laboratory Animal Toxicity Tests

An assessment of health risks associated with exposures of a general environmental nature requires prediction of effects from low level exposures of lifetime duration. Carcinogenic risks effects from environmental exposures must normally be estimated from animal data obtained at much higher levels because of the difficulty in detecting a small increase in tumor induction resulting from long-term low level exposure. Because the carcinogenic process is generally believed to be irreversible, self-replicating, and often originating from a single somatic cell mutation, assumptions of threshold levels of effect are believed to be invalid for many, if not all, cancer-causative compounds. Although many models have been proposed for extrapolation from animal data to human risk assessment, the one utilized here was chosen to facilitate uniform treatment of the variety of chemical compounds that are discussed in the development of those water criterion documents that deal with animal carcinogens.

It is recognized that the process of evaluating existing studies and resultant data in preparation for application of mathematical methods involves a high level of professional judgment. Many questions will necessarily arise due to the unique characteristics of the specific compounds under

discussion and the tremendous variability in completeness and comparability among the available studies.

A general explanation of the evaluation and extrapolation procedures to be used are as follows:

1. Since the compounds discussed are known, or suspect carcinogens, emphasis was placed on those studies with carcinogenic or mutagenic endpoints. In particular, those studies dealing with mammalian species.
2. The extrapolation method employed is a mathematical procedure that uses a single dose and observed response of a toxicologic experiment to estimate a dose level for humans that will not increase the risk of tumors by more than a specified level (1 in 100,000) (Personal communication, Dr. Todd Thorsland, CAG, U.S.EPA, Washington, D.C.). Clearly this method is predicated on sound toxicologic test procedures. Hence, each included study was evaluated for adherence to sound toxicological and statistical principles.
3. Judgment was exercised in prioritizing the significance of toxicologic studies that use different routes of administration. In general, the preferred route of exposure is oral (food, water, or gavage) followed by intraperitoneal, intravenous, inhalation, or dermal routes of administration for the same species. However, in some instances, consideration of absorption rates required that other routes be evaluated.

The NCI's Ad Hoc committee on the Evaluation of Low Levels of Environmental Chemical Carcinogens outlined two conditions that would render the extrapolations of animal carcinogenesis to man inappropriate. This committee reported to the Surgeon General as follows:

> Any substance which is shown conclusively to cause tumors in animals should be considered carcinogenic and therefore a potential hazard for man. Exceptions should be considered only where the carcinogenic effect is clearly shown the results from physical rather than chemical induction or where the route of administration is shown to be grossly inappropriate in terms of conceivable human exposure.

4. After selection of the sound toxicologic studies that form the basis for development of a recommended criteria, a single dose and observed response were selected for the most "sensitive" sex (if both males and females were tested) according to the following method: Select the lowest dose that yields a tumor response rate that is greater than the control rate. If the standard controls and media control response rates are not significantly different ($\alpha < 0.05$), a combined rate was calculated from controls.
5. The extrapolation methods were applied independently to each selected dose and response pair. The lowest projected dose was selected as the "safe level" based on the available toxicologic studies, if judgment indicated equal confidence in the various dose-response pairs.
6. The calculated safe dose was evaluated along with the results from human studies to develop a recommended criteria.

Calculation of Estimated Safe Levels for Humans:

The specific data analyses performed along with required input data are described in Mathematical Description of Extrapolation Method. This model provides the additional risk associated with ingestion of 2 liters of water per day and contaminated aquatic foods. Any other risks associated with air, food, or other exposure are not addressed by this model. A copy of the working data sheet is also included.

Mathematical Description of Extrapolation Method

A. Necessary information:

nt = No. of animals (males or females) exposed to selected dose that developed tumors (all sites combined unless tumors appear to be related to route of administration, e.g., peritoneal tumors would not be included if intraperitoneal injection method is used).

Nt = Total number of animals (male or females) exposed to selected dose level.

nc = Number of control animals (males or females) with tumors.

NC = Total number of control animals (males or females).

Le = Actual maximum lifespan for test animals.

le = Length of exposure (no. of hours, days, weeks, etc.).

d = Average dose per unit of time (mg/kg).

w = Average weight of test animals (kg).

B. Necessary information from general literature:

70 kg = Average weight of man.

L = Theoretical average length of life for test species, unless specified in articles. (See attached table for appropriate values.)

F = Average weight of fish consumed per day, assumed 18.7 grams.

C. Necessary ecological information.

R = Bioaccumulation factor for edible portions of fish (supplied by Environmental Research Laboratory, Duluth)

(Note: If a bioaccumulation factor is provided for the total fish or for some part other than the total edible portion (such as the fat) an attempt should be made to estimate factor for edible portion.)

D. Mathematical Model:

$$Pt = Pc + (1-Pc) \quad 1 - e^{-t^3 \sum D}$$

Where:

Pt = nt - NT = Proportion of test animals with tumors.

Pc = nc - NC = Proportion of control animals with tumors.

$D = \frac{d \times le}{Le}$ = Lifespan weighted average dose level (mg/kg)/(unit of Time)

$$B = \left\{ -\ln\left[\frac{1 - Pt}{1 - Pc}\right] - \right\}\left[D \times t^3\right] \text{ where } t = \frac{\text{lifespan for test animals}}{\text{length of life for species}} = \frac{Le}{L}$$

$B' = B\sqrt[3]{\frac{70}{w}}$ (Note: It is assumed that average weight of man = 70 kg

If and only if B' 0.1, then

$SL = \frac{10^{-5} \times 70}{-B'(2 + RxF)}$ = Safe level (mg/l) for man

If B' $\geq$ 0.1, then

$SL = \frac{\ln (1 - 10^{-5})}{-B' (2 + RxF)} \times 70$ = Safe level (mg/l) for man

(Note: It is assumed average daily consumption of water is 2 liters/day)

APPENDIX II

1. Calculation of Daily Occupational Exposure level of 2,4-Dinitrotoluene based on its Threshold Limit Value-Time Weighted Average (TLV-TWA) concentration (Am. Conf. Gov. Ind. Hyg. 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

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

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

$$= \underline{5.4\ mg}$$

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 5.5 as determined for the blue-gill sunfish (U.S.EPA report, Duluth, Minnesota),

b) Average weight of aquatic organisms consumed per day is 18.7 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

Bioaccumulation factor of 2,4-DNT = 5.5

The concentration of 2,4-DNT in fish =

740 x 5.5 x 0.0187 = 76 ng from aquatic organisms

Daily intake of

2,4-DNT from 2 liters

of drinking water = 740 ng/l x 2 = 1480 ng

Total intake/day = 1480 + 76 mg
or 1556 ng
(1.55 µg or .00155 mg)

APPENDIX III

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 B6C3F1 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 ($p = .01$) 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 200 ppm 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 740 ng/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 200 ppm in the diet. The time-weighted average dose of 45 mg/kg/day was given in the feed for 24 months, with the surviving animals sacrificed one month later. The mammary tumor incidence was 11/23 and 33/35 in the control and treated groups, respectively. The incidence of hepatocellular carcinomas and neoplastic nodules was 0/23 and 24/34 in the control and treated groups, respectively. Assuming a fish bioconcentration factor of 5.5, the criterion is calculated from the following parameters:

n_{t} mammary = 33	d = 45 mg/kg/day
N_{t} mammary = 35	R = 5.5
n_{c} mammary = 11	L = 25 months
N_{c} mammary = 23	W = 0.464 kg
n_{t} liver = 24	F = 0.0187 kg/day
N_{t} liver = 34	
n_{c} liver = 0	
N_{c} liver = 23	
le = 24 months	
Le = 25 months	

Based on these parameters, the one-hit slope, ^{B}H, is 2.95×10^{-1} for mammary tumors and 1.53×10^{-1} for hepatocellular carcinomas and hepatocellular neoplastic nodules. The resulting water concentration of 2,4-dinitrotoluene calculated to keep the individual lifetime cancer risk below 10^{-5} is 740 ng/l.