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OCCUPATIONAL HAZARD ASSESSMENT
OF TRINITROTOLUENE
Interim Report

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16. Abstract (Limit: 200 words) The biological effects of exposure to trinitrotoluene (118967) (TNT) were discussed, along with the evaluation and control of exposure. Exposure to TNT can occur during the production, handling, and packing operations. TNT has been used in textile dyeing and as the principal raw material in manufacturing phloroglucinol (108736), used in black on white photocopying. Metabolism follows one or more of three general pathways: nitroreduction, oxidation of an allyl group when present, and direct hydroxylation of the aromatic ring. Fatalities have resulted from aplastic anemia, toxic hepatitis, and other factors. Neurologic effects were noted which included excessive perspiration, acrocyanosis, transient eruption of red skin patches, unstable pulse and arterial pressure, and central nervous system disorders including headaches, dizziness, irritability, poor memory, and disturbed sleep. Ocular effects were described as well as dermal effects. Results of epidemiologic studies were highlighted. Sampling and analytical methods were reviewed, and methods for controlling safety hazards and health hazards associated with TNT were considered.			
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I. INTRODUCTION

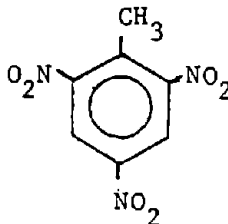
Properties

Trinitrotoluene (TNT) has six isomeric forms: 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-, and 3,4,6-TNT. However, 2,4,6-TNT is the form of commercial importance in the United States.

(a) Identification

TNT may be identified as follows:

- (1) Synonyms: 1-methyl-2,4,6-trinitrobenzene; TNT-tolite, tolit(e), trilit (French); 2,4,6-trinitoluol (German); trojnitroto (Polish); and trinitrotolyolum (Russian)
- (2) Chemical Abstracts Service (CAS) registry number: 118-96-7
- (3) Formula: $C_7H_5N_3O_6$
- (4) Structural formula



- (5) Molecular weight: 227.1

(b) Chemical and Physical Properties

The chemical and physical properties of TNT are listed below.

- (1) Appearance at 20 C: yellow and orthorhombic (crystalline form)
- (2) Boiling point at 99,975 Pa (750 mm Hg): explodes at 240 C
- (3) Melting point: 82 C
- (4) Specific gravity: 1.654
- (5) Vapor pressure at 85 C: 7.065 Ps (0.053 mm Hg)

(6) Solubility (g/100 g) at 20 C: acetone, 109.00; alcohol, 1.23; benzene, 67.00; carbon tetrachloride, 0.65; ether, 3.29; and water, 0.01.

(7) Explosive characteristics

TNT is one of the most stable high explosives. However, it rapidly releases energy and creates high pressures when detonated by strong shock (an impact equivalent to a 2-kg weight falling 130 cm), rapid heating or heating to 240 C, or when burned in large quantities or in a confined space [699].

(c) Synthesis

Manufacture of TNT is generally carried out in a closed, continuous process and sometimes in a closed, batch process in three steps, each involving the addition of a mixture of concentrated nitric and sulfuric acids. The process is exothermic and must be cooled. Figure I-1 shows the major steps in TNT synthesis. After the process is complete, the TNT is washed with sodium bisulfate to remove the asymmetric isomers that form in small amounts.

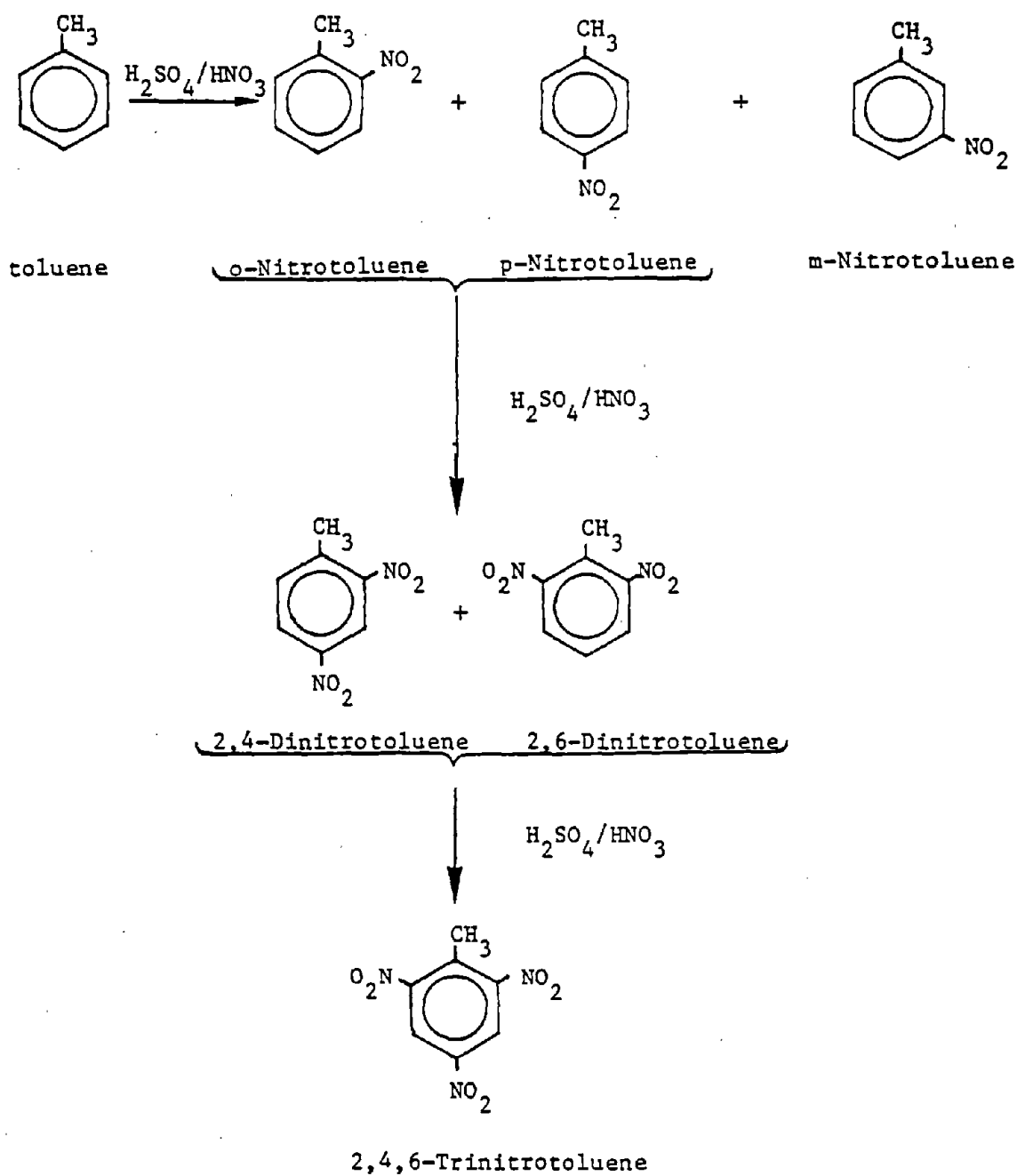


FIGURE I-1. SYNTHESIS OF 2,4,6-TNT

II. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Exposure to trinitrotoluene (TNT) can occur during production, handling, and packing operations. Manufacturing capability exists at four contractor-operated Army ammunition plants [479], which have a combined monthly capacity of 57 million pounds (25.8 Gg) of TNT. This capacity is not currently being utilized, however. In 1976, US production of 2,4,6-TNT, which is the principal constituent of TNT explosives and comprises 99% of military grade TNT [6], was estimated at 46 million pounds (20.9 Gg). This production level has declined rapidly. As of mid-1978, no facilities of the US Department of Defense (DOD) were actively manufacturing TNT [479,592,593,594,595,596]. Instead, existing stockpiles of TNT are being used for munitions maintenance. This application often involves melting the TNT and refilling it, creating potential exposure hazards. TNT may also be used in textile dyeing and as the principal raw material in the manufacture of phloroglucinol, which is used in black-on-white photocopying.

General Toxicity of Nitroaromatic Compounds

Trinitrotoluene (TNT) is a member of a group called nitroaromatic compounds. This group shares a number of common characteristics with regard to metabolism, methemoglobin formation, and carcinogenicity.

(a) Metabolism

Metabolism of nitroaromatic compounds can follow one or more of three general pathways: nitroreduction, oxidation of an alkyl group when present, and direct hydroxylation of the aromatic ring.

(1) Nitroreduction

Reduction of the nitro group occurs in three sequential steps: nitro to nitroso, to hydroxylamine, to amine [807], as shown in Figure 1:

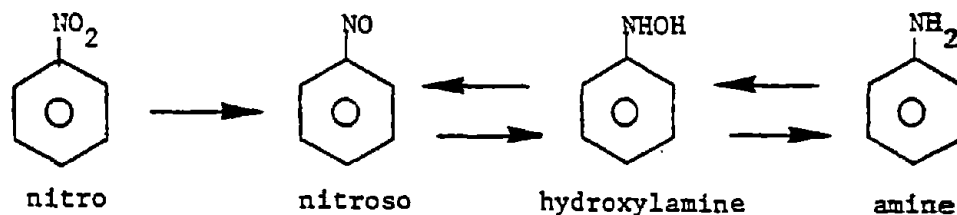


Figure 1.

Several nitroreductase systems have been described, especially in the liver [813,815]. In vitro reduction of nitrobenzene has been shown to be mediated by cytochrome P-450 [814]. Although cytochrome P-450 is usually thought of as an oxidative enzyme, the electron flow within the system appears to be reversible [808]. The reductive reactions of cytochrome P-450 in vivo may be much more important than presently assumed from in vitro reactions since the partial pressure of oxygen within the liver cell is much lower than that of air saturated aqueous solutions used in in vitro assays [808].

The reaction from nitroso to hydroxylamine to amine is reversible. N-oxidation has been shown to occur quite readily [808]. N-oxidation of the hydroxylamine and nitroreduction of the nitroso compound can also occur via reactions with hemoglobin (see section on Methemoglobinemia). Another possible source of nitroreduction is the gastro-intestinal bacterial flora. Treatment of germ-free animals with nitrobenzene did not result in methemoglobinemia whereas methemoglobinemia did occur following treatment of non germ-free animals [816]. The gut contents were shown to be the major source of nitroreductase in the non germ-free animals [816]. This indicates that following reduction by the gut-flora, reduced metabolites are absorbed into the circulation, where further metabolism and/or reactions can occur.

(2) Sidechain oxidation

Methyl groups on the aromatic ring can be sequentially oxidized in the following manner in Figure 2:

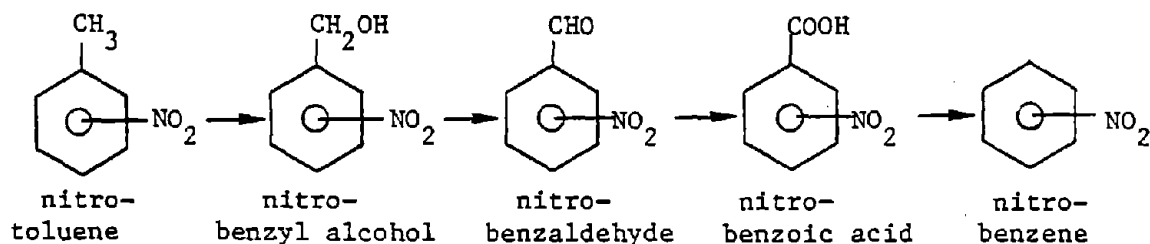


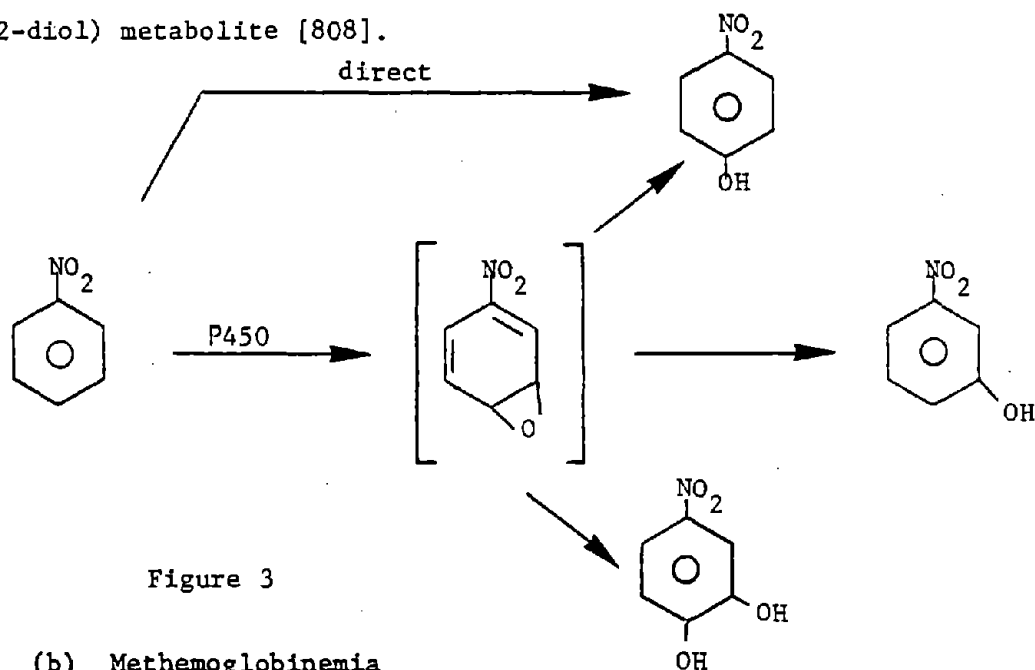
Figure 2.

Either an aromatic carboxylic acid or the demethylated product results [804]. These reactions appear to be mediated by cytochrome P-450 [808]. Some end products as well as some of the intermediates were identified as metabolites for the nitrotoluenes (see section on Metabolism). Metabolism of 2,4,6-TNT resulted in 1,3,-diamino-5-nitrobenzene, indicating that demethylation does occur in the metabolism of 2,4,6-TNT [623].

(Error in Paging)

(3) Aromatic hydroxylation

Hydroxylation of aromatic rings can occur in at least two fashions, direct hydroxylation [804] or cytochrome P-450 mediated epoxidation [808] (Figure 3). In the latter reaction, the intermediate epoxide can rearrange to two isomeric phenolic metabolites or can be hydrated to a catechol (1,2-diol) metabolite [808].



(b) Methemoglobinemia

Methemoglobinemia is defined as a clinically increased level of circulating methemoglobin or more properly ferrihemoglobin [110]. Methemoglobin results when one of the four Fe^{2+} atoms in hemoglobin is oxidized to Fe^{3+} [110]. Methemoglobin cannot bind oxygen; it can however be reconverted into hemoglobin enzymatically through a diaphorase mediated reaction [110]. Conversion of methemoglobin or hemoglobin to sulfhemoglobin (via an unelucidated mechanism) results in the irreversible loss of hemoglobin from the blood [810]. Methemoglobin, always present in the blood in small quantities, can result from autooxidation and dietary sources, such as nitrite and nitrate [110]. Congenital methemoglobinemia has also been reported; most of the cases are due to a NADH diaphorase deficiency [810]. Infants are more sensitive to methemoglobin formation, as they have a low

NADH-diaphorase activity [810]. Sensitivity to methemoglobinemia also occurs in people deficient in glucose-6-phosphatedehydrogenase (G-6-PD). People deficient in G-6-PD (11% of the American Black males are affected) are unable to reduce NADP^+ to NADPH at a normal rate [810]. Table VI-9 shows the incidence of G-6-PD deficiency in several races [801]. A side effect of G-6-PD deficiency is the increased formation of methemoglobin and Heinz bodies following administration of certain oxidative drugs through an unidentified mechanism [810]. G-6-PD deficiency also results in decreased levels of reduced glutathione (GSH). GSH is important in the maintenance of membrane integrity and can also be used to reduce methemoglobin [810].

(1) Mechanism of formation and reduction of methemoglobin

The essential reaction in the formation of methemoglobin during the metabolism of an aromatic nitro compound is the reduction of the nitroso compound to the hydroxyl amine which in turn is oxidized via a reaction with oxyhemoglobin to the nitroso compound which in turn is again reduced to the hydroxylamine. This sets up an enzymatic cycle, resulting in the oxidation of many Fe^{2+} atoms to Fe^{3+} by one equivalent of nitroso compound resulting from oxidation of the hydroxylamine [110]. Although this statement adequately reflects the overall observations, the mechanism used to describe this cycle appears faulty. Kiese [110] describes the simultaneous oxidation of phenylhydroxylamine to nitrosobenzene, and of ferrohemoglobin to ferrihemoglobin (methemoglobin) (see Figure 4):

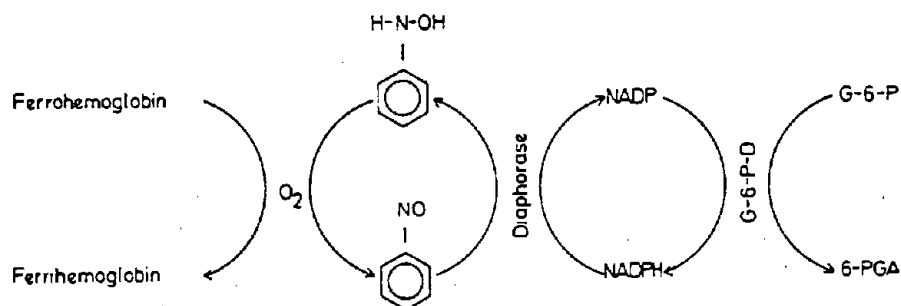
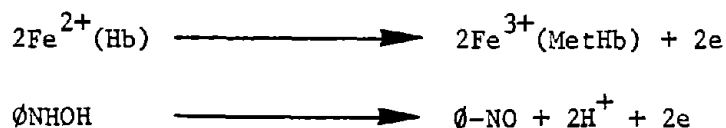


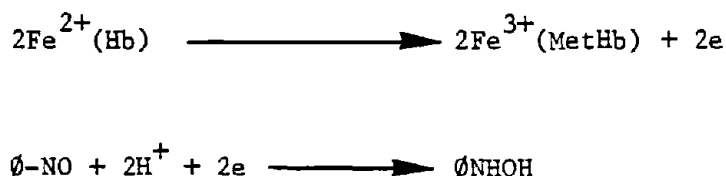
FIGURE 29. Enzymic cycle of ferrihemoglobin formation in red blood cells by arylhydroxylamines and nitrosoarenes.

Figure 4.

This reaction does not appear feasible because it involves the simultaneous oxidation (liberation of electrons) of both substrates in the reaction.



A more appropriate scheme describing the production of methemoglobin would be an oxidation-reduction process or:



The latter mechanism was shown to occur for Fe^{2+} porphyrins in a model system [811]. Enzymatic methemoglobin reduction has elsewhere been reported to be NADH dependent rather than NADPH dependent as claimed by Kiese [110,810]. A NADPH dependent diaphorase does exist, but only functions in the presence of artificial electron carriers such as methylene blue [810] which is commonly used in the treatment of acute methemoglobinemia [810]. Nonenzymatic methemoglobin reduction via reduced glutathione (GSH) and ascorbic acid (Vitamin C) normally accounts for only a small portion of methemoglobin reduction [810]. However, ascorbic acid is successfully used in the treatment of chronic (i.e. congenital) methemoglobinemia [810].

An alternative reaction to that presented by Kiese describing the formation and reduction of methemoglobin is depicted in Figure 5.

The nitrosocompound reacts with hemoglobin to produce the hydroxylamine and methemoglobin (a redox reaction). Methemoglobin can be reduced to reform hemoglobin using NADH dependent diaphorase. The diaphorase in Figure 5. gets its reducing equivalent via cytochrome b_5 and NADH from glycolysis. The hydroxyl amine can be oxidized back to the nitrosocompound using either hemoglobin or cytochrome P-450 as the oxygen carrier.

(c) Carcinogenicity

N-hydroxylation of aromatic amines leading to the formation of hydroxyl amines is thought to be the first step in the formation of the proximate carcinogens [812]. Reductive metabolism of the aromatic nitrocompounds also leads to the formation of hydroxylamines (see Metabolism). Further activation of the hydroxylamines is achieved via the formation of the N-hydroxyesters [812].

Thus the proximate carcinogens of aromatic nitrocompounds are formed via the reduction of the nitro group through a nitroso to a hydroxyl amine followed by subsequent acetylation [809].

Hydroxylamine derivatives are much more potent carcinogens than either their parent nitro- or amine compounds [809]. A very good indication of the similarity of the proximate carcinogen, i.e., the N-hydroxylaminoester, of both amino- and nitro derivatives is that both amino and nitro compounds will cause carcinogenesis at the same sites [809].

Occupational standards that prevent methemoglobinemia may not necessarily prevent against cancer since the reduction of methemoglobin levels induced by nitroaromatic compounds below the clinical level (1%) [810], does not

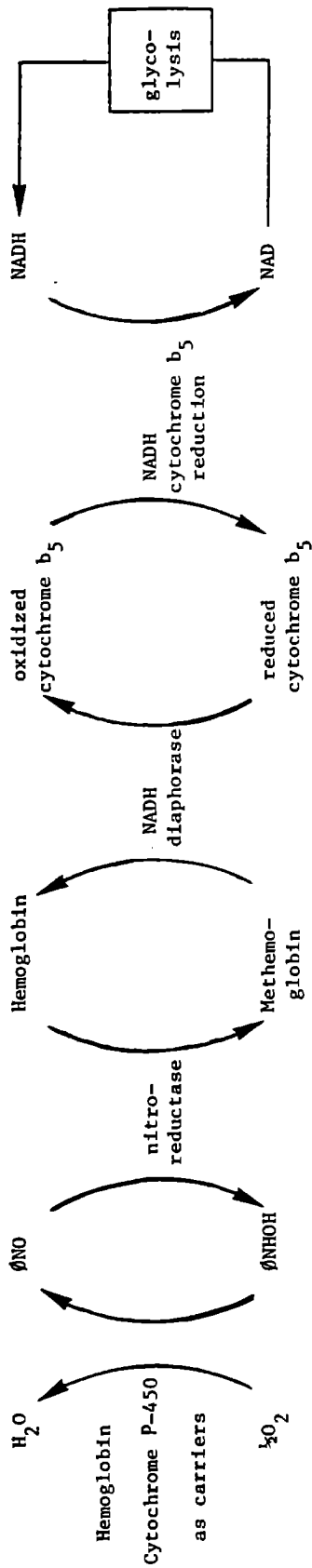


Figure 5.

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result in the disappearance of hydroxylamines from the body. A threshold level for carcinogenic response has never been established; therefore, any amount of hydroxylamines in the body might result in the eventual development of cancer.

Historical Reports

TNT was first synthesized in 1863 by Wilbrand [61]. However, the production of TNT on an industrial scale did not begin until about 1890 in Germany [542]. The first military application was in 1905 during the Russo-Japanese War [8]. By 1910, TNT had attained worldwide use in munitions operations, largely replacing picric acid [61]. Until then, it was considered to be relatively nonhazardous [496,526]. This opinion was supported by

White and Hay [495] in 1901, when they reported the effects of hypodermically injecting a cat with 60 drops (approximately 5.3 cm^3) of a 1% solution of the substance. The authors noted only slight signs of cyanosis and concluded that TNT was relatively harmless to cats when compared with nitrobenzene, which can cause death in cats, when similarly dosed, in a few hours.

During World War I (WWI), there was an increase in the frequency of illnesses among TNT handlers [526,629]. In 1915, fatalities resulting from TNT intoxication in England were also reported [502]. The first cases of fatal TNT poisoning in the United States were reported between 1916 and 1917 [629]. An estimated 17,000 cases of TNT intoxication, resulting in 475 fatalities, were reported in the United States within a 7.5-month period during WWI [65]. In contrast, only 22 fatalities were reported from all government-owned explosives plants during the 4-year period of WWII. McConnell and Flinn [65] attributed the decrease to improved industrial hygiene programs.

During 1916-17, a total of 370 cases of TNT poisoning with 96 fatalities occurred in England. After the 1917 deaths, researchers recognized the need to clearly define the signs of exposure to TNT to prevent further fatalities.

In 1919, Voegtlin et al [485] reported that dogs administered oral (po) doses of 5-33 mg/kg TNT developed cyanosis, anemia, and digestive disturbance, and then died. These same investigators [485] were also interested in the effect of diet and duration of exposure in different species of animals and in man. They noted that some persons were either not adversely affected by, or were able to recover from, any effects associated with exposure to TNT. In 1917, Martland [629] cited a British Ministry of Munitions summary

of TNT poisonings to support his conclusions. Only 50 cases of fatal toxic jaundice were reported among the many thousands of workers exposed to TNT, so he concluded that a small minority of workers are susceptible and liable to develop this jaundice. Several investigators discussed the possibility that some persons were predisposed due to age [597,629], sex [267,526,597, 630], race [267,476], dietary or drinking habits [476,598], or previous illnesses including anemia [476,598] and hepatic [476,598], respiratory [598,629] or kidney [267] disease. Often, however, healthy workers were also adversely affected by exposure to TNT [598,599,609]. One investigator reported that sex was not a significant factor [267]. Investigators in both the United States and England found that age did affect susceptibility to TNT poisoning [267,597,629].

The influences of the route of entry and environmental conditions during exposure on the incidence of TNT poisoning were also investigated in efforts to prevent illness and death. A condition such as high temperatures in the work environment causes workers to dress lightly and allows more areas of the body to be exposed. In addition, oily skin, because TNT is in contact with the skin longer [476], as well as chafing of the skin, enhances chemical absorption through the skin [597]. The relative importance of the route of administration was controversial [476,506,597]. Some investigators believed the dermal route to be the most important in humans or animals [506,598,626]. Others considered inhalation an important route [267,506]. Accidental ingestion was also recognized as a probable mode of exposure to TNT that could result in adverse effects [506,597]. Oral toxicity was demonstrated in guinea pigs and rabbits in 1920 [506].

Effects on Humans

Although a frequent cause of dermatitis, TNT was generally believed to be non-toxic until 1915, when attention was called to cases of poisoning in Britain [65]. In the United States, there were 475 deaths among 17,000 cases of TNT poisoning within the first 7.5 months of WWI. From June 1, 1941, to September 1, 1945, there were 22 deaths reported from Government plants that processed 95% of US explosives during WWII [65].

Studies reporting the toxic effects of TNT in humans have described fatalities due to aplastic anemia [5,7,65,74,471], toxic hepatitis [65], and other effects such as methemoglobinemia with cyanosis [65], neurologic disturbances [17,604], cataracts [422,464], skin rashes [72,598,606,622], and inflammation of the reproductive tract [166].

(a) Aplastic Anemia and Other Effects on Blood

In 1946, McConnell and Flinn [65] reported the records of 22 deaths from TNT that occurred from June 1, 1941, to September 1, 1945. Of these 22 deaths, 8 were attributed to toxic hepatitis, 13 to aplastic anemia, and 1 to either aplastic anemia alone or in combination with toxic hepatitis. The number of nonlethal cases of toxic jaundice and aplastic anemia was not known, but very few persons with aplastic anemia recovered from this disease.

The average age at death from aplastic anemia was 45 years, the median period of exposure was 216 days, and the time from onset of definite symptoms to death ranged from 6 to 185 days with a median figure of 40 days. The early symptoms were weakness, loss of appetite and weight, mild cough, and bleeding from the nose and gums. In several instances, symptoms were absent or the person noticed only a few purple spots on the body. Rapid reduction

of erythrocyte count and hemoglobin concentration were the most conspicuous signs of significant bone marrow damage.

Only 33% of the 22 persons who died were exposed to average concentrations in excess of 1.5 mg of TNT/m³ of air, and a few of these probably were respirators. Many unaffected workers were similarly exposed. The authors believed that the amount of TNT ingested or absorbed through the skin depended largely on the hygiene and personal habits of the individual. Susceptibility appeared to be influenced by personal factors of general health and nourishment.

Hart et al [5] and Eddy [7], in 1944, and Sievers et al [74], in 1946, reported eight cases of aplastic anemia similar to those previously described [65]. Seven persons had died, but the eighth individual, the only female, was still alive at the time of the 1946 report. Exposure, in most cases, was in bomb-loading facilities. Atmospheric concentrations were reported as ranging from 0.5 to 10.7 mg/m³, but sampling and analytical methods were not specified. The total population similarly exposed was not mentioned, and the wearing of a respiratory protective device was recorded for only one case. Only two individuals were known to have taken daily showers; thus, skin contamination was likely. Table II-1 summarizes the cases.

Eddy [7] previously considered that so-called aplastic anemia in TNT workers was likely to be the final stage of hemolysis by TNT. However, the cases reported in this study showed the hypoplastic or aplastic bone marrow typical of aplastic anemia. In all the cited reports [5,7,74], the significance of absorption of TNT through the skin was apparently not appreciated.

TABLE II-1
CASES OF APLASTIC ANEMIA FOLLOWING
EXPOSURE TO TNT

Sex	Age	Atmospheric* Concentration mg/m ³	Duration of Exposure	Death	Reference
M	26	Not reported	Several mo	Yes	7
M	43	2.1-3.4	24 mo	Yes	74
M	43	4.2 3.1 1.1	4 d 26 d 46 d	Yes	74
M	50	1.1-7.0	1.5 mo	Yes	5,7
M	50	3.7-10.7	11 mo	Yes	5
**M	55	5.4-7.0	1 mo	Yes	5
M	65	4.2	4 mo	Yes	5,7
F	32	0.5	30 mo	No	74

* Sampling and analytical methods not defined

** This individual used a dust respirator

In 1954, Crawford [471] recorded all cases of toxic anemia and toxic purpura due to TNT that were noted in the United Kingdom (UK) during WWII. Of the 24 subjects diagnosed as having aplastic anemia, 9 persons recovered and 15 died. The author considered that early effects of TNT on the circulating blood, or hemolysis, methemoglobinemia, and sulfhemoglobinemia, could be compensated by TNT stimulation of the bone marrow to hyperplasia. However, with further intoxication the bone marrow fails; all its blood-forming elements are affected to variable degrees. The clinical manifestations of the reported cases included hemorrhage from various sites, such as gums, nose, retina, and conjunctiva, including two cases of menorrhagia. The anemia was often severe and usually normocytic or macrocytic. Evidence of liver damage was found in 10 of the 15 persons who died. Of the nine patients who recovered, three showed jaundice at some time. The author noted that toxic aplastic anemia had a most insidious onset, and emphasized that the expected signs and symptoms of pallor, fatigue, giddiness, breathlessness on exertion, and abdominal pain might be appreciated only at a very late stage. Cyanosis was noted only in six persons, and only one showed typical anilism. This report is a useful record of the clinical course of TNT aplastic anemia, but says nothing about exposure beyond the fact that weapons were largely filled with TNT by hand.

Ermakov et al [604] examined 574 individuals in the USSR who were occupationally exposed for 6-25 years to TNT at concentrations in air slightly exceeding the maximum permissible concentration of 1 mg/m^3 . In 22.2% of the subjects, normochromic anemia and leukopenia with relative lymphocytosis was demonstrated, and 50% had slight thrombocytopenia. This report did not mention the age, sex, or occupation of the subjects. It did not state any

criteria of diagnosis, and no control population was compared. Therefore, it contributes little to the evaluation of the effect of TNT on the blood.

In 1945, Stewart and coworkers [606] examined some of the early effects of TNT exposure in 62 healthy volunteer students who worked in melting and filling operations in a munitions factory (see Table II-2). The students worked for 4-11 weeks for 8 hours/day, 5-6 days/week. Area TNT concentrations ranged from 0.3 to 1.3 mg/m³. After cessation of exposure, the preexposure concentration of Hb and the number of circulating red blood cells (RBC's) significantly decreased ($P < 0.01$), and the preexposure count of reticulocytes significantly increased ($P < 0.01$) in 85% of the subjects. More pronounced hemolysis was observed in males than in females. All groups showed slight but significant increases ($P < 0.01$) in the average level of total serum bilirubin after exposure. In 44% of the subjects, a rash of varying intensity appeared on the hands. There were four cases of transient cyanosis and one case of severe cyanosis. The students wore overalls and caps; however, they worked with bare hands and did not wear any respiratory protection, so dermal, respiratory, and oral exposures occurred. The authors indicated that the exposure period was too short to follow the initial reactions to exposure to TNT to a stable condition. This careful study provides useful information on the biologic effects of TNT during the early period of exposure. In healthy young adults, TNT appears to act on the liver less rapidly than on the blood.

In 1974, El Ghawabi [8] examined the biologic effects of TNT on 38 workers with average exposure of 9.9 years in a TNT plant. The findings were compared with those from 20 workers in a control group having similar distributions of age and socioeconomic status. The author indicated that the control group

TABLE II-2

SUMMARY OF SIGNS AND SYMPTOMS

IN VOLUNTEERS EXPOSED TO TNT

Students		Exposure		Rash		Number of Cases (Percent)		Weight (kg)	
No	Sex	Operation	Max/Min (days)	Severe-3 (9%)	Moderate-5 (11%)	TNT Sickness (Hands only)	Cyanosis	Misc**	Before/After
Group 1	44 F	Filling	56/9	Severe-3 (9%)	Moderate-5 (11%)	Severe-2 (5%)	Severe-0	16 (36%)	64.9/61.5
					Slight-14 (32%)	Moderate-10 (23%)	Transient-0		
						Slight-12 (27%)			
Group 2	8 M	"	54/20	Severe-1 (12%)	Moderate-0 (8%)	Severe-0	Severe-0	3 (37.5%)	63.5/61.3
					Slight-4 (50%)	Moderate-0	Transient-0		
						Slight-4 (50%)			
Group 3	10 M	"	31/8	Severe-0	Moderate-0	Severe-0	Severe-1 (10%)	10 (100%)	64.9/64.9
		Melting	30/3	Slight-0	Moderate-2 (20%)	Moderate-2 (20%)	Transient-4 (40%)		
					Slight-5 (50%)	Slight-5 (50%)			

†TNT sickness includes vomiting, abdominal pain, nausea, and diarrhea.

**Miscellaneous symptoms include nasal and throat irritation, fatigue, menstrual disturbances, and edema.

Adapted from Reference 606

was far from the site of exposure before and during the time of the study. There was a significantly lower Hb concentration ($P < 0.05$) in the exposed group than in the control group, but there were no significant differences in liver function or other hematologic tests (see Tables II-3 and II-4). Mean area concentrations of TNT in the different workplaces varied from 0.1 to 1.2 mg/m³. In the crushing area, a site of intermittent operations, concentrations were found to range from 2 to 10 mg/m³. Higher TNT concentrations were measured in the air during the summer than during the winter. The exposed group had a higher incidence of symptoms such as sneezing, sore throat, coughing, and stomach ache than the control group. Clinical examinations of exposed workers revealed no cyanosis, no cases of cataract, and no dermatitis. Many details were omitted from this report on important aspects of hygiene to avoid skin contact and on medical selections and rejections, labor turnover, etc. Thus, it is difficult to account for the apparent absence of more significant health effects and biologic changes.

Brill et al [608] and Cone [523] reported transitory initial leukocytosis accompanied by moderate eosinophilia in all 17 of a group of TNT plant workers who were exposed to TNT at area-sampled concentrations averaging 2.0 mg/m³ during 8-hour daily workshifts over a 5.5-month period. Eight weekly blood counts, followed by four monthly counts, were done on stained blood smears. The presence of toxic granulations and any abnormalities in size or shape were noted, but reticulocyte and platelet counts were not done. Prior to the study, all the workers were exposed to TNT for a consecutive period of at least 6 months, and most were exposed for 1 year or longer. Previously instituted precautionary measures included a daily change of clothing and compulsory showers for the workers, as well as routine

TABLE II-3
INCIDENCE OF SYMPTOMS IN TNT-EXPOSED
WORKERS AND CONTROL GROUPS

Symptom	*		**	
	-----Exposed Group-----		-----Control Group-----	
	Number of	Percent	Number of	Percent
	workers	of	workers	of
	with	Total	with	Total
	Symptoms		Symptoms	
Sneezing	21	60.0	1	5
Sore throat	18	51.4	1	5
Cough	16	45.7	4	20
Stomach ache	15	42.8	1	5
Loss of appetite	10	28.4	2	10
Constipation	13	37.14	2	10
Flatulence	12	34.28	3	15
Headache	4	11.43	2	10
Lassitude	2	5.71	1	5
Nausea and vomiting	12	34.28	1	5

Adapted from reference 8

* Exposed Group (n=35). Three workers were excluded because of past history of bilharziasis, viral hepatitis, and diabetes mellitus.

** Control Group (n=20)

TABLE II-4

BLOOD AND LIVER FUNCTION TESTS IN WORKERS

EXPOSED TO TNT AND IN A CONTROL GROUP

	-----Exposed Group-----		-----Control Group-----	
	Range	Mean	Range	Mean
Blood				
Hematologic Studies				
Hb	73-100	85.171%	92-100	96%
RBC's	3,470,000	4,381,140/mm ³	1,680,000	4,200,000/mm ³
	5,200,000		5,109,000	
Total leucocytic count	3,100-9,400	5,984/mm ³	3,200-10,500	5,800/mm ³
Basophils	0-1	0.57%	0-2	0.7%
Eosinophils	0-6	2%	0-7	2.1%
Staf polymorphs	0-9	3.3%	0-6	3.2%
Segmented polymorphs	45-70	57.74%	50-72	59.3%
Lymphocytes	22-40	31.75%	2-41	31.9%
Monocytes	0-6	3.10%	0-7	3%
Liver Function Tests				
Thymol turbidity test	2-7	3.9 units	1-8	3.4 units
Zinc sulfate turbidity	6-12	8.2 units	4-10	7.9 units
Bilirubin	0.4-1.1	0.8 mg/100 ml	0.3-1	0.7 mg/100 ml
Alkaline phosphatase	6-11	7.8 K A units	4-10	6.9 K A units
Serum glutamic-oxaloacetic transaminase (SGOT)	6-14	8.7 units	4-13	8.9 units
Serum glutamic-pyruvic transaminase (SGPT)	3-13	7.3 units	2-10	6.2 units

Adapted from reference A

area monitoring for TNT. The results indicated that exposure to TNT at the concentrations studied had little effect on the formed elements of the blood if precautionary measures are taken. However, the evidence appears to contradict that of other investigators who have found deleterious effects on the blood from exposure to TNT at a level of 2.0 mg/m^3 and below. The processes and details of medical surveillance were not sufficiently described to assess the true exposure to TNT or to be confident that all health effects were noted. The erythrocyte and leukocyte counts were erratic, whereas the Hb estimations were incredibly uniform, even considering the admittedly approximate Tallquist method. Data from the blood and liver fraction tests are presented in Table II-4.

In 1961, Prikolotin [465] reported the results of a study on 106 persons working in constant, direct contact with TNT. No means of personal hygiene were used. Area concentrations of TNT averaged 1.4 mg/m^3 with a maximum of 8.4 mg/m^3 . The mouth and skin were assumed by the author to be the principal routes of exposure because of low concentrations of TNT dust in the air. Laboratory tests were conducted to determine the amount of TNT in the blood. The investigator found that 31.3% of the men and 66.7% of the women probably had detectable concentrations of TNT in the blood, as measured by the excretion of sodium benzoate in the urine. The author concluded that women were not more sensitive to TNT, but intoxication began earlier in women because their skin is thinner and less hair-covered than that of men. He also stated that all ages were equally susceptible to TNT intoxication. Both Hb concentrations and the number of erythrocytes were markedly depressed in the workers exposed longer; enlarged livers were also found in 15% of these women and 9% of these men. The author attributed the complaints of headaches

and weariness in 20% of all workers to the action of TNT on the central nervous system. In this and other ways, the author appears to confirm findings with theories that might be acceptable in the USSR, but not in the West.

(b) Hepatic Effects

McConnell and Flinn [65], in the study reported previously, noted that hepatitis occurred more often than aplastic anemia in younger persons (average age 30) and after shorter exposure periods (average 63 days). Early symptoms, such as nausea, vomiting, drowsiness, and general malaise, were indefinite. In some instances, the person continued to work until jaundice appeared or the icterus index was found to be raised. Cyanosis was frequently observed, but the blood picture was usually unchanged. The time period from the first definite sign or symptom to death ranged from 12 to 53 days. These comparisons suggest that aplastic anemia and toxic hepatitis are separate pathological states to which different individuals in a group similarly exposed to TNT may succumb.

Palmer et al [474] noted toxic lesions of the liver as one of the rarer manifestations of TNT poisoning and reported three cases of hepatic necrosis. Necrosis, as indicated by the intensity and duration of jaundice, was accompanied by decreased concentrations in the blood of cholesterol esters and prothrombin and by increased excretion in the urine of hippuric acid and galactose. These cases involved a female, aged 22, and two males, aged 46 and 63. All worked in an ordnance plant, where they had been exposed as long as 6 months to TNT dust or fumes or to clothing contaminated with TNT. All individuals recovered completely from the effects of TNT. No information was given of exposure levels or conditions of work. These are clinical

accounts of toxic hepatitis in which marked jaundice was accompanied by totally normal blood cell counts and Hb concentrations.

In 1975, Marchenko et al [152] reported the results of an investigation of 167 workers in a TNT production plant. An unmatched group of 160 unexposed workers served as controls. Of the exposed workers, 80% were women 20-45 years of age. Of these women, 40% had been employed in TNT production for at least 5 years, and 60% had worked for more than 10 years. Several measures of hepatic function were evaluated. Significant increases in the concentrations of bilirubin and serum beta-lipo-proteins were found among the exposed workers, thereby indicating functional liver impairment.

In the study by Prikolotin [465], sex-linked differences in the incidence of liver dysfunction, as indicated by Quick's hippuric acid test, were also seen: men 36.4%, women 49.8%. In this test, the uninjured liver parenchyma eliminate 70-80% of administered sodium benzoate; a drop below 60% indicates liver dysfunction. The "full chronic intoxication" of the liver, which was not defined by the author, was apparent in 33.2% of the women and in 18.2% of the men. Further sex-linked differences were observed in the physical examination, in which 15% of the women and 9% of the men were found to have enlarged livers. These were the same individuals who eliminated only 37-50% of administered sodium benzoate and showed strong Webster's reactions. No age-dependent differences were reported.

(c) Neurologic Effects

In the study by Ermakov and associates [604] reported earlier, long-term TNT intoxication was found in 122 of 574 individuals who were occupationally exposed to TNT. The majority of these 122 subjects exhibited autonomic disorders including excessive perspiration, acro-cyanosis, transient

eruption of red skin patches, unstable pulse and arterial pressure, and central nervous system (CNS) disorders, such as headaches, dizziness, irritability, poor memory, and disturbed sleep. It is difficult to accept that all the subjects' symptoms and signs were due only to TNT exposure because no control group was used.

(d) Ocular Effects

Tyukina [422] reported in 1967 cataract formation induced by exposure to TNT in which changes in the crystalline lens occurred within 5-6 months in persons who experienced so-called significant contact with TNT dust or vapor. Exposure that was characterized as involving less contact with TNT produced signs of cataracts in 2-3 years, according to the author. Women exposed to significant contact developed cataracts within 6 years, while men with similar exposures developed cataracts within an average of 2-3 years. This difference suggested to the authors that women might be less susceptible than men. Because no control group was used in this study, it is not possible to ascribe the observed effects to TNT. The stages in the development of cataract were detailed, but the author did not relate cataract development to levels of exposure or to other signs or symptoms of TNT toxicity.

In 1971, Zakharova and Manoilova [469] reported the examinations of 360 persons, aged 29-52 years, who were occupationally exposed to TNT for 5 or more years. They detected eye cataracts in 163 of these persons. Reduced visual activity was found in 65 persons, but this was attributed to cataracts in only 25 persons. The authors concluded that changes in the lens and other parts of the eye were characteristics of poisoning by low doses of TNT. Most of the 163 subjects complained of headache, poor sleep, tiring easily, and irritability, or a greater tendency to perspire, or

they showed nervous system or gastrointestinal signs or symptoms. No details of exposure levels or work practices were provided. The lack of a control group in this study makes it difficult to attribute the observed effects solely to TNT exposure. Chronic gastritis, astheno-autonomic syndrome, and other biologic departures from normal were reported in the absence of marked changes in the blood and liver; the clinical or prognostic significance to the health of the workers is difficult to assess.

Melnichenko [150], in 1976, reported the health effects of exposure by nine blasters to explosives containing TNT. The two job categories of loader and blaster were considered. The concentrations of TNT poured from bags ranged from 21 to 90%. Sampling and analytical methods were not specified, no information on the other constituents was given, and the total number of individuals in the study was not reported. No control group was used in the study.

Loaders had TNT in the washwater from their hands within 1 hour after their workshift began, and TNT was present in the blasters' respiratory zones during 35% of the workshift. During this time, holes were drilled and filled with granulite for blasting that contained 21% TNT in the form of scales. Pouring this material caused granules to surround the blasters' breathing zones. Concentrations of TNT in the workers' respiratory area ranged from 0.65 ± 0.12 to $12.51 \pm 3.31 \text{ mg/m}^3$. However, the author did not report a time-weighted average (TWA) exposure level. Cataracts described as being produced by TNT were found in 12.9% of the subjects. In addition, various unspecified changes in the nervous system, liver, gastrointestinal tract, and blood were observed in 17% of a randomly selected group of loaders and in 18.3% of a group of blasters.

Loaders and blasters were not occupationally exposed to TNT during all of their workshifts. However, the apparently work-related effects seen in these workers are similar to effects that are specific to TNT poisoning [653]. Working conditions were the main concern of this study, so health effects were not reported in detail. Cataracts were reported by Zakharova et al [469], Tyukina [422], and Melnichenko [150]. Ermakov et al [604] reported that 20% of the 574 individuals developed cataracts after being exposed to concentrations of TNT that slightly exceeded the 0.001 mg/liter maximum permissible concentration (MPC) standard. Most of these individuals were exposed to the hazard for 6-25 years.

Cataract formation resulting from exposure to TNT has been reported only in uncontrolled studies performed in Eastern Europe. No such effect has been reported in Western literature, although researchers attempted to find such effects.

(e) Dermal Effects

In 1916, Livingston-Learmonth and Cunningham [598] reported a study on an unspecified number of women at a munitions factory who handled TNT powder or who were exposed to fine particles of TNT in the air. No figures were given, but the authors discussed the common occurrence of skin rashes, especially among newer workers. These rashes occurred on the hands, face, and neck, and also on the thighs and feet of workers with poor personal hygiene. Rashes were also found around the mouths of workers who wore respirators that were infrequently cleaned. The rashes were intensely itchy, particularly in persons who perspired excessively.

In another study published in 1916, White [622] described six individuals, aged 20-45 years, who were workers in mixing rooms at munitions factories.

They complained of, and were treated for, intensely itching or burning dermatitis that sometimes appeared within 1 week of contact with TNT. Most of these individuals handled fine TNT powder in one of the mixing processes, such as sieving. Of six reported cases, five were in males and one was in a female, aged 20. Rashes were observed on the skin of the hands, fingers, arms, thighs, feet, abdomen, legs, face, and genital regions. In every case, the earliest manifestation, on the exposed skin closest to the TNT, was a superficial erythema in which the patches tended to coalesce. When irritated, the skin tended to thicken and produce vesicles that ruptured. In no cases were the hands of workers affected. Descriptions of TNT dermatitis were detailed, and the author again stressed the aggravation of dermatitis by perspiration.

Stewart and coworkers [606], whose study is described in detail under blood effects, observed that a rash appeared on the hands of 27 (44%) of 62 student volunteers who worked in TNT melting and filling operations in a munitions factory. These students worked with bare hands for periods ranging from 4 to 11 weeks for 8 hours/day, 5-6 days/week, in areas where environmental concentrations of TNT ranged from 0.3 to 1.3 mg/m³. These reports are not definitive, but they indicate that contamination of the skin by TNT, especially as fine powder, can cause dermatitis. The effect can become severe if the dermal area is further irritated, or if the person perspires excessively or has oily skin.

Epidemiologic Studies

With the advent of WWII, the US Government, the major domestic manufacturer of TNT, instituted a program to reduce worker exposure to TNT and to prevent fatalities due to TNT. The program included establishing appropriate work practices and engineering controls such as local exhaust ventilation; educating workers, medical personnel, and management on the hazards of TNT; and having industrial hygienists and safety experts make frequent workplace inspections [413]. From June 1941 to September 1946, a total of 22 fatal cases of TNT poisoning were reported [65,413] from government-owned ordnance explosives plants, which produced 95% of all military explosives made in the United States. During that period, the annual occupational disease fatality rates were 40/100,000 for people working with TNT and 3/100,000 for all workers at those plants. The causes of death in the TNT workers were listed as toxic hepatitis (8), aplastic anemia (13) and toxic hepatitis and aplastic anemia (1). The average age of the 8 who died of hepatitis was 33 years, 6 (75%) of whom had been exposed for less than 3 months. The average age of the 13 who died of aplastic anemia was 45 years, of whom only 1 (7.1%) had been exposed for less than 3 months. Of the 22 who died, 15 were exposed at average environmental concentrations of $\text{TNT} < 1.5 \text{ mg/m}^3$, according to the study. He did not stipulate, however, whether these TNT concentrations were obtained by personal or area monitoring. He also did not discuss to what degree, if any, skin absorption of TNT contributed to the deaths. Therefore, the actual TNT exposures that caused these deaths are uncertain.

In 1976, Morton and coworkers [81,264] reported effects resulting from exposure to TNT at concentrations below the present Federal limit of 1.5 mg/m^3 . In order to minimize absorption of the substance, the TNT facilities studied required the use of powder uniforms, headcovers, gloves, clean underclothing,

daily showers, double lockers in change rooms, handwashing before meals, local exhaust ventilation, and NIOSH-approved respirators for dusts and vapors during steam cleaning operations. Eating was not allowed in the work area. Concentration of hemoglobin (Hb) and activities of lactic dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT) in the blood were estimated for 45 workers on a TNT pack-and-fill line monthly from December 1973 through April 1974. Using paired "t" tests, the authors compared concentrations of Hb and activities of LDH and SGOT obtained at atmospheric concentrations of TNT of 0.3 mg/m^3 with those obtained at the 0.8 mg/m^3 level. Differences in activities of LDH were statistically significant at $P < 0.005$ and those for SGOT were statistically significant at $P < 0.01$. Differences in Hb concentrations were not significant. Nearly all abnormalities in the concentration of Hb and the activities of SGOT and LDH in the blood discovered by the authors occurred in March and April, corresponding to exposures of 0.8 and 0.6 mg/m^3 , respectively. In March, 59% of the workers had abnormally high activities of LDH in their sera, and 23% of these also had abnormally high activities of SGOT. One worker had an elevated activity of SGOT only. Normal values in this study were: Hb, 13.5-16 g/100 ml (Sahli method); LDH, 50-100 units (Wacker method [806]); SGOT, 8-45 units (Reitman and Frankel procedure [805]). When the employees were removed from exposure to TNT, the LDH activities returned to the normal range in 1-3 weeks. The recovery time for employees with elevated SGOT activities was not ascertained because of the variability in values. Although LDH and SGOT activities decreased when exposure was discontinued, the actual values had not returned to the preplacement levels when the facility was closed in April 1974, after which employees were not examined.

These results suggest that definite effects on the liver, appearing as elevated activities of LDH and SGOT in serum, occurred after exposure to atmospheric TNT at concentrations of approximately 0.6-0.8 mg/m³ (see Table II-5). The authors stated that TNT exposure was due primarily to dust. They did not specify the type of sampling train used, so it is not possible to determine if TNT vapor was collected as well as aerosol. Thus, there is some question regarding the stated exposure concentrations. Early detection of these subclinical changes should provide a much greater margin of protection for the worker. At the 0.3 mg/m³ level, no effects were observed on LDH and SGOT activities. This does not rule out the possibility that activities of these enzymes could be affected by long-term exposure to TNT at this concentration. The authors also stratified the data according to age, sex, and race, but the small number of individuals in several categories prevented drawing any conclusions regarding differences in susceptibility to the toxic effects of TNT.

The demonstrated biologic effects resulting from exposure to TNT at concentrations below the present Federal limit of 1.5 mg/m³ prompted a study [494] by the US Army Environmental Hygiene Agency (USAEHA) to ascertain safe levels for TNT. Initiated in 1975, this cross-section epidemiologic study included workers in five army ammunition plants. A total of 626 employees exposed to TNT and cyclotrimethylene trinitramine (RDX), and 865 unexposed workers, agreed to participate after being randomly selected from the entire exposed and unexposed populations of the plants. These subjects constituted 73.7% of all workers asked to participate in the study. Some statistically significant differences in response rates were detected between the exposed and unexposed populations in some plants, but the overall response rate was not significantly different for the two groups.

Initially, the variation in response rates from plant to plant caused some concern, since bias in worker selection could occur. For example, sick employees exposed at relatively high concentrations of TNT responded at high rates, and those exposed at low concentrations responded at low rates. However, analysis of the data revealed that these differences appeared to be related to specific administrative problems at the respective plants. Consequently, the authors concluded that participants in the study were representative of the total work force at the five plants. Nevertheless, they did not present evidence to conclusively rule out selection bias.

Blood samples from participants were analyzed for SGOT, LDH, Hb, serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, bilirubin (total and direct), albumin, glucose, cholesterol, blood urea nitrogen (BUN), total protein, hematocrit (Hct), reticulocyte count, and fluorescent antinuclear antibodies (FANA). Area airborne exposures to TNT ranged from below 0.01 to 1.84 mg/m³, the 8-hour time-weighted averages (TWA's). Airborne samples were collected using a glass fiber filter. However, a filter will not collect TNT vapors, so the sampling method used in this study cannot accurately determine the TNT exposures of the workers. The health questionnaire completed by the subjects indicated to the authors that requirements for the various jobs controlled dermal exposure.

Although worker exposure to RDX occurred in conjunction with exposure to TNT, the investigators ruled out bias of the data by RDX. This conclusion was made after the USAEHA reviewed a study by Hathaway and Buck [495], which showed that workers exposed to RDX at concentration of 1.57 mg/m³ or less exhibited no measureable effects on blood when compared with controls. However, this conclusion does not take into account the possible synergistic or antagonistic effects that may result with simultaneous exposure to TNT and RDX.

TABLE II-5
MEAN TNT DUST LEVELS, PRODUCTION RATE AND
MEAN LABORATORY TEST VALUES
OF EMPLOYEES EXPOSED TO TNT BY MONTH

	Preemployment	12/73	1/74	2/74	3/74	4/74
Hb	15.58 (±1.15)*	14.96 (±0.72)	15.19 (±0.88)	15.07 (±0.99)	14.85 (±1.11)	14.90 (±0.99)
SGOT	34.78 (±18.15)	35.11 (±16.59)	37.47 (±18.77)	39.06 (±37.06)	58.17 (±70.73)	54.44 (±50.87)
LDH	64.37 (±16.52)	52.63 (±12.16)	51.72 (±11.57)	50.88 (±11.02)	105.78 (±39.68)	102.76 (±40.91)
Production Rate (% Capacity)	N/A	75	75	75	126	100
Average TNT Dust Levels	N/A	0.3 mg/m ³	0.3 mg/m ³	0.3 mg/m ³	0.8 mg/m ³	0.6 mg/m ³

* (±) = Standard deviation about the mean.

Normal values: Hb = 13.5-16 g/100 ml; LDH = 50-100 units; and SGOT = 8-45 units

Adapted from reference 81

The effects of different atmospheric concentrations of TNT on the Hb concentration in blood can be seen in Tables II-6 and II-7. Table II-6 shows a general increase in the relative odds of developing a subnormal concentration of Hb with increasing environmental concentrations of TNT in comparison with unexposed workers. Although this was not pointed out by the authors, $0.10\text{--}0.19\text{ mg/m}^3$ was the lowest concentration at which a statistically significant ($P<0.01$) number of subnormal Hb concentrations occurred. Below this exposure level, the numbers of workers with subnormal Hb concentrations in the exposed and unexposed groups were not statistically different.

Table II-7 presents the mean Hb concentrations in the blood of male and female participants in the study [494] as the average concentrations of TNT in the air of the workplace increased. For males, the data revealed decreasing mean Hb concentrations with increasing levels of TNT exposure. The slight anomalies in Hb concentration occurring in categories 6 and 7 can be accounted for by the relatively small number of individuals in each category. For females, there was no apparent relationship between increasing environmental concentrations of TNT and decreasing concentrations of Hb in the blood. In all categories, there were smaller numbers of females than males, which may have contributed to the disparity between male and female results. It has not been possible to compare the means of the Hb concentrations in the exposed and the unexposed groups statistically, since the standard deviations of the means were not given by the authors. From this study, it can be concluded that workers exposed to TNT at atmospheric concentrations as low as $0.1\text{--}0.19\text{ mg/m}^3$ may experience a reduction in their Hb concentrations to values below the normal range.

TABLE II-6
RELATIONSHIP BETWEEN TNT EXPOSURE AND
NUMBER OF WORKERS WITH NORMAL/ABNORMAL
Hb CONCENTRATIONS

TNT Exposure Concentration (mg/m ³)	Number "Abnormal"*	Number Normal	Relative Odds**
None	78	785	1.0
<0.01	24	191	1.3
0.02-0.09	10	63	1.6
0.10-0.19	18	80	2.3
0.20-0.29	4	20	2.0
0.30-0.39	5	5	10.1
0.40-0.49	14	33	4.3
0.50-0.99	11	21	5.3
1.00-1.49	3	5	6.1
1.50+	1	1	10.1

*Males: "abnormal" is less than 14 g Hb/100 ml blood.

Females: "abnormal" is less than 12 g Hb/100 ml blood.

**An estimate of relative risk of "abnormal" Hb with increasing exposure to TNT. In all cases, the odds are relative to those not exposed.

Example: 0.10-0.19: $RO = \frac{18 \times 785}{78 \times 80} = 2.3$

Adapted from reference 494

TABLE II-7
RELATIONSHIP BETWEEN MEAN HEMOGLOBIN CONCENTRATIONS
IN BLOOD AND CONCENTRATIONS OF TNT IN AIR
FOR MALES AND FEMALES

Category	TNT Concentration (mg/m ³)	Males		Females	
		Mean Hb	Number of Participants	Mean Hb	Number of Participants
1	None	15.2	602	13.5	261
2	<0.01	14.9	154	13.2	66
3	0.02-0.09	14.7	46	13.5	27
4	0.10-0.19	14.6	71	13.2	27
5	0.20-0.29	14.3	17	13.8	7
6	0.30-0.39	13.9	9	10.6	1
7	0.40-0.49	14.4	30	13.1	17
8	0.50-0.99	14.2	24	13.3	9
9	1.00-1.49	13.7	8	0	-
10	1.50-1.84	13.1	1	12.4	1

Adapted from reference 494

In 1974, Friedlander et al [605] described the health effects of TNT exposure in workers in a TNT wash-out facility. The study was initiated because of unspecified symptomatology and blood abnormalities noted in the workers. Atmospheric TNT concentration, taken in three areas, ranged from <0.02 to 4.0 mg/m^3 . The use of respirators began only 2-3 weeks prior to the study. Personal hygiene consisted of daily showers after work and daily changes of coveralls and gloves. The authors noted that some employees having contact with TNT did not wear gloves.

In the first part of the study, the Hb and Hct concentrations of 12 employees were measured before and after assignment to the TNT facility. Statistically significant decreases ($P<0.01$), measured by a paired "t" test, were observed in both physiological parameters. Clinical significance in the form of anemia was also observed, since 10 of the 12 employees had Hb concentrations below normal laboratory limits.

In the second part of the study, more detailed hematologic and blood chemistry data were obtained from 24 employees in the facility and a control group of 20 non-TNT-exposed workers of similar age, sex, and seniority from other areas of the Army Depot. Abnormal laboratory test results were more frequent in the TNT-exposed group than in the control group, and occurred only in the exposed group for Hb, Hct, red blood cell count, reticulocyte count, and renal function measurements. The mean values for these tests, as well as for glucose, in TNT-exposed workers differed significantly ($P<0.05$) from those of the controls.

The data presented in this study do not permit a determination of the actual TNT concentration at which the observed effects occurred for the following reasons: the TNT samples were taken when the plant was temporarily activated for sampling, and test values do not necessarily reflect the levels present during the actual exposure period; the extent to which contact

contributed to the total TNT exposure was not quantified; and collecting TNT on a glass fiber filter, a procedure used for 33% of the samples, has been shown to underestimate the TNT concentration. Thus, the data are not useful for determining a safe level for TNT exposure.

Animal Toxicity

(a) Short-Term Toxicity Studies

Lee and coworkers [441] administered 4.12% TNT (purity >99%) in peanut oil by intragastric intubation to mice and observed convulsions lasting for about 2 hours. The animals were observed for 14 days. Within 5-15 minutes after dosing, the animals exhibited ataxia, but recovered completely in 24-48 hours. The LD₅₀'s were approximately 1 g/kg for each sex. No gross pathology due to treatment was noted in animals that died. In a second study, the same concentration of TNT in peanut oil was given by the same route to rats. LD₅₀'s of 1.01g/kg in males and 8.20 g/kg in females were estimated. These animals, like the mice, had convulsions lasting for up to 2 hours. Those that survived had transient cyanosis and ataxia.

A 4.12% solution of TNT in peanut oil was topically applied to 16 guinea pigs. In four of the guinea pigs, a moderate sensitization response was obtained, using the Magnusson and Kligman maximization test [505]. A paste of 50% TNT in peanut oil was also applied to intact and abraded skin of six rabbits. The reaction for both treatments was scored as a mild irritation according to the Draize method described in 21 CFR Part 191.11. A red stain was also apparent at the site of treatment. The authors observed no eye irritation 24 and 72 hours after application of a paste of 50% TNT in peanut oil to 6 rabbits, although a red stain was apparent around the eyes.

Reports of porphyrinuria in workers exposed to nitro compounds prompted Somers and West [503], in 1944, to compare the effects of administering TNT by subcutaneous injection (sc) and by stomach tube to groups of three adult

albino male rats on a porphyrin-free diet. The test animals weighed 150-200 g each. Doses of 200-600 mg/kg of TNT suspended in 2% mucilage of acacia in tap water were administered once every 2 days for 18 days by both routes. Doses higher than 400 mg/kg of TNT more than doubled the mean total urinary excretion of porphyrin, compared with that before administration of the TNT or with that of the controls, which were treated with a corresponding sc dose of mucilage of acacia only. In addition, liver damage of an unspecified nature and enlarged stomachs were seen in the animals injected sc with 600 mg/kg TNT. The authors concluded that, because Webster's test for TNT in the urine was negative throughout the study in both groups of animals, porphyrinuria, which indicates a disturbance of pigment metabolism, was not related to the presence of TNT in the urine. These results, however, do not account for possible age, sex, or strain differences, because only male rats were tested. Furthermore, only small numbers of animals were tested. The results, therefore, must be regarded with qualifications.

Haythorn [506], in a 1920 report, administered TNT po, sc, intravenously (iv), and percutaneously to 28 guinea pigs and an unspecified number of rabbits. The doses were administered in oil, 10% alcohol, serum, normal salt solution, or lanolin. The amounts were not reported, except that relatively larger doses were given to the rabbits. The schedule and duration of the administration varied and could not be determined precisely. The researchers found cyanosis, anemia, myocardial depression, hypothermia, and hyperplasia of the bone marrow in both species. Liver lesions in the guinea pigs included total necrosis, fatty changes, and diffuse areas of necrosis; lungs were often edematous and hemorrhagic. The authors concluded: Liver lesions were similar to those in human cases cited in their report; severe anemia was hyperplastic

in type; and removal of animals from exposure to TNT usually led to complete recovery. Unfortunately, the lack of specific data makes the author's conclusions questionable.

Von Bredow and Jung [88] investigated the effects of 2,4,6-TNT in producing methemoglobin in 12 cats given ip doses of 0.02-0.08 g/kg. The results showed increases in methemoglobin in all animals ranging from 33 to 88% of the Hb total, peaking 3 hours after the injection, and returning to the pretreatment value after 120 hours. There was a clear linear response between the dose of TNT and the amount of methemoglobin formed. One mole of TNT formed approximately 1-7 equivalents of methemoglobin. In addition, nervous signs, including paralysis of the rear limbs, decreased temperature, and narrowed pupils, were seen only at doses of 40 mg/kg and greater. Anemia was seen in all the animals, and the formation of Heinz bodies after treatment varied from 3 to 40 times that of the pretreatment values. Two cats died after 3.5 and 5.5 hours after being given 0.15 and 0.10 g/kg of TNT.

In 1974, Mul'menko and Levina [71] reported the action of TNT on serotonin, which causes constriction of the blood vessels. Male white rats, weighing 259-275 g, received technical grade TNT internally once a day at 100 mg/kg in a suspension of liquid starch. The dose corresponded to 33% of the single lethal dose given by this route of administration. One group received the TNT for 3 days, a second group received it for 30 days, and a control group received only the liquid starch. The serotonin levels in the brain and liver tissue decreased after only three doses of TNT. The level of serotonin in the liver was reduced by more than 50% compared to the values of the control animals; for the reduction of the serotonin level in the brain was much less pronounced. Although the total TNT dose was equal to a single LD₅₀ dose, none of the rats died. No significant changes in their general condition or

behavior during the time of poisoning were noted. However, the authors did note that TNT toxicity was accompanied by a disturbance in pyridoxine metabolism, although they specified neither the disturbance nor the method of assessing it. Pyridoxine enters into tryptophan metabolism, eventually leading to serotonin synthesis. Thus, the reduction of serotonin levels could be related to disturbances in pyridoxine metabolism.

(b) Long-Term Toxicity Studies

Hart [243] administered 1.0, 0.1, or 0.02 mg/kg TNT daily for 13 weeks by gastric intubation to groups of three male and three female Rhesus monkeys, aged 44-52 weeks. A 10% decrease in body weight occurred in the last week of the study in all the animals, including the controls. However, the animals receiving the TNT did not regain as much weight as the controls by the end of the study. At the end of 13 weeks, two of six animals receiving the highest dose had greater hemosiderin deposits in the cytoplasm of the liver cord cells than did the control animals. No other abnormalities were observed. Ranking the amount of hemosiderin deposits on a scale of one (no deposits) to five (many deposits), these two animals were ranked as two and four. Animals at the low and middle doses were not ranked.

Himsworth and Glynn [486] mixed 150 mg/kg of TNT into each of three diets, with 60% protein, 90% carbohydrate, and 50% fat, and fed an unspecified number of Wistar rats for up to 6 weeks. Equal numbers of control animals were fed each diet. The investigators reported that the rats fed TNT in the protein diet gained weight at the same rate as before the addition of the TNT.

In rats on the carbohydrate diet, no significant weight loss was observed but a reduction in the normal weight gain was recorded. In contrast, the animals on the the fat diet lost weight rapidly and progressively; by day 100, their weight was 29% below that of the control animals. In addition, livers

of the animals on the fat diet showed fatty infiltration and necrosis of the liver cells, and increased accumulation of fat in the bone marrow in which up to 40% of the cells were erythroblasts, increased polychromosia and reticulocytosis, leukocytosis, the appearance of normoblasts, siderosis of the spleen, and hair loss. A deep red pigment was also observed in the urines of rats on the fat diet. By decreasing the fat content of the diet in several stages to 7%, a decrease in the red color in the urine was noted. Except for the liver lesions and hair loss, the same effects were also seen to a lesser degree in the animals on the protein and carbohydrate diet. The number of animals was not specified, but these results indicate that susceptibility to TNT was modified by the nature of the diet. Necrosis of the liver and anemia can apparently be produced by TNT in rats fed a diet high in fat content. It is possible, however, that a factor other than the fat content of the diet is the decisive circumstance leading to these toxic effects.

Foulerton [484] administered TNT to three cats and two rabbits. One cat received 236 mg/kg in the diet in two unequal doses during 2 days; another had a total dose of 2,533 mg/kg applied to its flank in vaseline for 17 days; and a third was given "occasional" doses of 143 mg/kg in the diet during 17 days. One rabbit was intubated 21 times with varying doses of TNT totaling 3,752 mg/kg during 30 days. A second rabbit was injected sc with TNT in a solution with saline with varying doses totaling 2,317 mg/kg in 13 days. Both rabbits and the cat that received occasional doses were killed by the experimenter; the other two cats died during the course of the studies. The results in the two animals were similar. These included blackened, concentrated urine; interlobular round-cell infiltration of the liver; fat in the liver and the cortical portion of the kidneys; and dark brown pigment in the spleen. The number of animals studied was very small, but the effects

produced within the two species appeared to be sufficiently uniform to suggest a general pattern of toxic action.

In a 1916 report, Kramer and Meierhof [491] described administration of unspecified amounts of TNT po, sc, and topically to dogs. Subsequently, they observed that the dogs developed diarrhea, weakness, emaciation, and leukocytosis. Necropsies revealed degeneration of the liver cells and congestion of the capillaries around the efferent veins in the liver. In addition, Hb was found lying free in bone marrow, the lymph nodes, and the spleen. In 1919, Voegtlin [485] reported administering up to 33 mg/kg of TNT to dogs po for an unspecified period of time and noted cyanosis, salivation, diarrhea, incoordination, jaundice, anemia, weight loss, and death.

In 1944, Donahue and coworkers [517] reported giving up to 100 mg/kg TNT in the diets of two dogs, 6 times a week for 4 weeks. During the first 10 days, the authors observed confusion, incoordination, irregular eye movements, diarrhea, and dark urine. By the end of the 2nd week, the effects on these animals appeared to be greatly reduced.

Hart [255] gave up to 1 mg/kg/day to three groups of six dogs for 90 days and observed no significant changes in appearance, behavior, blood chemistry, hematology, urinalysis, and gross and microscopic examination. Von Oettingen [468] exposed four tracheotomized dogs daily to vapor from 50 mg/kg TNT, 5 days/week for 2 weeks, and then to vapor from 100 mg/kg TNT for an additional 2 weeks. The appearance and blood analyses of the dogs were unchanged. In another study, Von Oettingen et al [518] administered by insufflation through the tracheal cannula up to 50 mg/kg TNT for 5 days/week for 17 weeks to groups of three animals. Recorded effects included weight loss, salivation, vomiting, diarrhea, weakness, incoordination, and moderate anemia.

In 1977, Dynnik et al [771] studied the effect of TNT on the condition of the kidney. Groups of three dogs were administered 0 or 7 mg/kg TNT (isomer unspecified) as a 7% oil solution sc twice weekly for 9 months. Significant decreases in renal plasma flow and tubular reabsorption were observed, amounting to 40-45% at the end of the experiment. Glomerular filtration was 1.4-2.1 times higher than the initial value during months 3 and 4, decreasing (unquantified) only in the last month of the experiment. The volume of glomerular filtration remained within the normal range. Pathological observations included a plethora of the vessels with signs of stasis and degeneration of tubular epithelium. In most of the proximal tubules, the epithelium showed granular degeneration and pronounced vacuolization. In some (unquantified) animals, more severe changes were observed: necrobiosis and death of the epithelium, or an extreme degree of cytoplasmic vacuolization with almost total dissociation of the cytoplasm. In addition to epithelial tubular changes, signs of interstitial edema and small clumps of lymphohistiocytic cellular elements, particularly around vessels, were observed in most of the animals.

In the above studies with monkeys [243], dogs [255,468,485,491,517], cats [484], and rabbits [484], toxic effects on the central nervous system (CNS), blood, and liver appeared by various routes of administration. However, small numbers of animals were used and the results were not uniform or predictable even in similar studies. Extrapolation of the data, except for generalities, is questionable.

Metabolism

(a) Absorption, Distribution, and Excretion

In 1979, El-hawari et al [800] described the metabolism of TNT as a function of the route of administration (oral, dermal, or intratracheal) in rats, mice, rabbits, and dogs. Groups of four Sprague-Dawley CD rats of each sex received oral (po) doses of 100 mg/kg of radioactively labeled TNT. After 24 hours, about 60% of the radioactivity was present in the urine, indicating massive conversion to polar metabolites, and 32% was present in the gastrointestinal (GI) tract and its contents. The only difference between sexes was in fecal radioactivity levels: 8% for males and 2% for females. Representative tissue levels for male rats at 24 hours were: blood, 2.9 (u)g/liter; liver, 10.7 (u)g/liter; kidney, 3.5 (u)g/liter; lung, 0.3 (u)g/liter; spleen, 1.8 (u)g/liter; and muscle, 0.8 (u)g/liter. These results indicate that accumulation of TNT-derived radioactivity occurred to any extent only in the liver. Levels for females were similar, but radioactivity levels in muscle tissue were 2.1 (u)g/liter, or 2.5 times higher than those of males. Following po or dermal dosing of 50 mg/kg of TNT of male CD rats, blood levels were lower in dermally exposed animals 4 and 8 hours after dosing, but blood levels were similar in all animals at 24 hours. Following dermal exposure of CD rats to 50 mg/kg of TNT, liver and kidney levels of radioactivity were lower than those following po exposure, but fat levels were higher. Recovery of radioactivity following the dermal dose was only 25% of that following the po dose. Thus, absorption of a dermal dose was less than that of an equal po dose, resulting in lower tissue levels except for adipose tissue, most of which is subcutaneous.

Inhalation exposure to TNT aerosols proved to be technically infeasible for these authors. A comparative study of tissue distribution was performed 4 hours after po or intratracheal administration of 50 mg/kg of TNT to CD rats, some of which had cannulated bile ducts. In previous comparative studies, tissue distribution was determined at 24 instead of 4 hours. Male rats dosed intratracheally had significantly ($p < 0.05$) higher levels of radioactivity in the blood, kidney, brain, muscle, fat, urine, and bile, compared with those of the other rats. Radioactivity levels in the liver, lung, and spleen were similar in male rats in both groups. Thus, intratracheal administration resulted in higher levels of radioactivity in the tissue. Recovery was only half that following po administration, but no explanation was provided by the authors. Possible explanations include exhalation, ciliary transport out of the lungs, and experimental error. The level of radioactivity in the GI tract and contents was significantly decreased by approximately 80% in intratracheally - vs po - dosed male rats. In the first group, most of the GI radioactivity (85%) was derived from bile secretion; in the second group, most of the GI radioactivity appeared to be nonabsorbed TNT, as only 20% of GI radioactivity is bile-derived. Biliary excretion was also higher following intratracheal administration.

After both po and intratracheal administration, radioactivity was present in the bile and the blood at 15 minutes. Following po administration, bile and blood radioactivity levels rose until 1 hour after dosage and then appeared to be steady or declined slowly. Following intratracheal administration, bile and blood radioactivity levels were approximately five times greater at 15 minutes, peaked at 30 minutes, declined rapidly, and then slowed after 2 hours. At 4 hours, both levels were 60%

higher in the intratracheally exposed male rats, compared with the other males. Thus, intratracheal administration resulted in a much faster uptake of TNT and, initially, at much higher blood levels.

Similar findings were reported for mice, rabbits, and dogs. Female mice had much lower blood, liver, kidney, GI, and fecal levels of radioactivity than male mice; there was only 60% recovery, compared with 80% for males. Following dermal administration of TNT to mice, the level of radioactivity in the liver decreased, and the level in the fat increased significantly, compared with po administration. Fecal, urine, and GI levels also decreased, and total recovery was only half that following po administration. Mice absorbed more TNT than rats following dermal administration and showed more sex-linked differences in metabolism, but the findings were generally similar to those for rats.

Administration po of 5 mg/kg of TNT to rabbits did not result in any sex-linked differences in tissue distribution. Dermal administration resulted in decreased levels of radioactivity in the blood, GI tract, and residual bile, but increased levels in the kidney, lung, brain, and fat. Recovery was 71% of that following po administration. Rabbits absorbed more TNT following dermal administration than rats, mice, or dogs and did not show any sex differences in metabolism. Other findings were similar to those for rats.

Following po administration of 5 mg/kg of TNT to dogs, no sex-linked differences in tissue distribution were observed. Dermal administration resulted in decreased levels in the blood, liver, kidney, spleen, muscle, GI tract, residual bile, feces, and urine, but increased levels in fat. Recovery was less than 20% of that observed following po dosing. Findings for dogs were similar to those for rats.

At equal dose levels, intratracheal administration resulted in faster absorption and distribution than did po administration, which resulted in faster kinetics than did dermal administration. There was a substantial amount of enterohepatic circulation for TNT, as evidenced by the TNT and its metabolites that were excreted via the bile and then absorbed from the GI tract. TNT appeared to be accumulated in the liver following a single exposure.

This study [800] has many drawbacks, such as the use of only one or two animals for some studies. (Single-animal studies are not discussed here.) Different end points were used for different routes; results of po vs intratracheal administration were measured at 4 hours, while po vs dermal results were measured at 24 hours. The data appear to be incomplete, as the total tissue levels of TNT do not equal the total recovery in any of the experiments.

(b) Identification of Metabolites

(1) Human Studies

As part of a 1945 study on TNT metabolism, Lemberg and Callaghan [623] described the urinary metabolites of TNT in two munition workers. Urine samples from both subjects tested positive for hydroxylamino derivatives. Other metabolites identified were 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, and two unidentified so-called aminonitrocresols, which could be amino/nitro derivatives of benzyl alcohol and benzoic acid. This study indicated that human metabolism of TNT includes both nitroreductive and oxidative metabolism.

(2) Animal Studies

In 1979, El-hawari et al [800] studied the biotransformation of TNT to urinary metabolites following po, dermal, or intratracheal administration of (ring-¹⁴C) 2,4,6-TNT to rats, mice, rabbits, and dogs. Urinary metabolites were analyzed using radiographic thin layer chromatography (TLC) of ethylacetate extracts from acidified urine. Conjugates were analyzed in urine treated with β -glucuronidase, which resulted in the liberation of TNT metabolites present as glucuronide conjugates. Following po dosing of male rats, approximately 34% of ethylacetate-extractable urinary metabolites were free or unconjugated; dermal dosing resulted in 63%. In rats with cannulated bile ducts, these amounts increased to 80% and 70%, respectively. Thus, biliary excretion appeared to account for a large percentage of the glucuronide adducts. Similar data were reported for male rabbits and dogs and female rats. However, for male mice, 80% of the ethylacetate-extractable urinary metabolites were found to be unconjugated.

The chemical characterization of metabolites in this study was incomplete: only comparative, radiographic TLC data were reported, and identification was thereby made by chromatography of reference compounds. The resolution of these TLC data was absolutely insufficient to allow for positive characterization. However, the metabolites "identified" were: 2,6-dinitro-4-hydroxylaminotoluene, 4,6-dinitro-2-hydroxylaminotoluene, 4,6-diamino-2-nitrotoluene, 2,6-diamino-4-nitrotoluene, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 2,4,6-trinitrobenzylalcohol, and 2,4,6-trinitrobenzoic acid. An azoxytoluene compound was also detected in some of the analyses, but the researchers suspected that it might have been an artifact resulting from the extraction procedure.

In a 1944 study by Channon et al [94] rabbits were dosed by gavage with TNT at various levels. The compounds 4-amino-2,6-dinitrotoluene, 6-amino-2,4-dinitrotoluene, and 2,6-dinitro-4-hydroxylaminotoluene were identified in the urine; several procedures were used, including determinations of the melting points of metabolites and their derivatives. Again, an azoxytoluene compound was detected, but it was shown to be an artifact resulting from storage and extraction techniques. Approximately 30% of the dose was excreted as 4-amino-2,6-dinitrotoluene. Large amounts of metabolites were excreted in the urine as glucuronide adducts, accounting for 47% of the total dose. Glucuronide excretion was complete within 48 hours. Trinitrobenzyl alcohol and its reductive metabolites also appeared to be TNT metabolites; a particular red coloration of the urine occurred only after administration of TNT or trinitrobenzyl alcohol.

In 1945, Lemberg and Callaghan [623] described the urinary metabolites of TNT in rats. Rats (unspecified) received approximately 1,000 ppm TNT in their daily diet, and their pooled 24-hour urine was analyzed. The compounds 4-amino-2,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 1,3-diamino-5-nitrobenzene were isolated as TNT metabolites. The substance 1,3-diamino-5-nitrobenzene was also observed as a urinary metabolite following administration of 2,4-diamino-6-nitrotoluene, indicating possible demethylation of TNT following reductive metabolism. No hydroxylamino derivatives could be detected. Unidentified metabolites of a "phenolic nature" were also detected. The authors assumed these to be aminonitrocresols. However, they could be nitro/amino derivatives of benzyl alcohol or benzoic acid, as they have been detected in other studies of TNT metabolism [94,800]. No specific analysis for such metabolites was performed in this study.

(c) Summary

TNT appears to be absorbed relatively rapidly, depending on the route of administration: intratracheal>oral>dermal. More than half of the absorbed dose was excreted in the urine as polar metabolites within 24 hours. A proposed metabolic scheme for TNT, represented in Figure II-2, is based on data from human, mouse, rat, rabbit, and dog studies. The relative importance of pathways in the different species is unknown, as a definitive study of TNT metabolism has not yet been reported. TNT appears to accumulate in the liver, the probable tissue of its metabolism, following a single exposure. Large amounts of TNT metabolites are secreted as glucuronide conjugates into the bile, then reabsorbed in the GI tract and, finally, excreted in the urine.

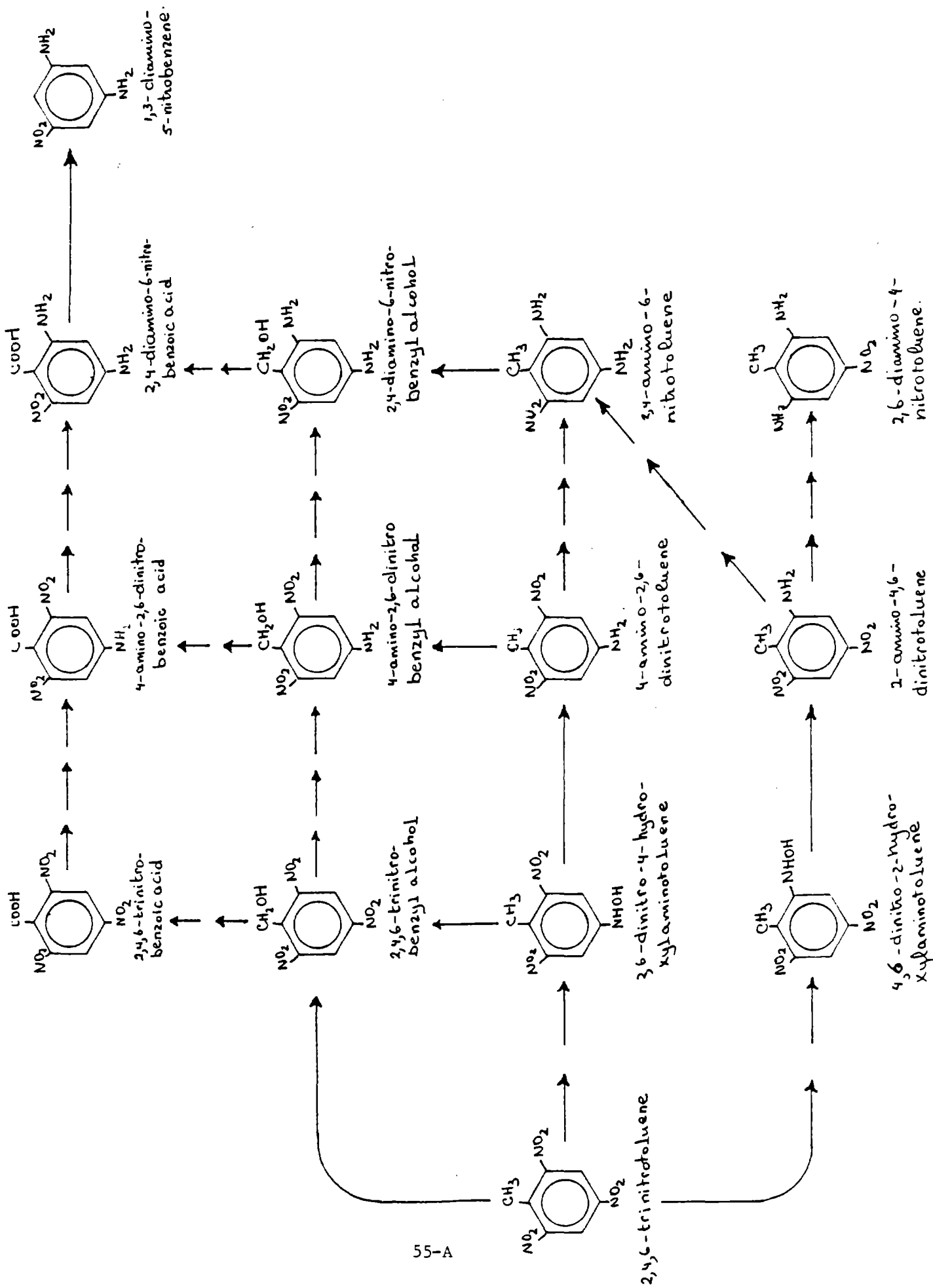


Figure II-2. Proposed metabolic pathways for 2,4,6-Trinitrotoluene.

Carcinogenicity, Mutagenicity, and Reproductive Effects

(a) Carcinogenicity

No studies on the carcinogenic effects of TNT were found. However, positive mutagenic responses for TNT in two Salmonella assays and one cytogenicity test, and hydroxylamines, probable precursors of proximate carcinogens, were identified in the metabolism of TNT. These results warrant precautions in the use of TNT. The studies are discussed under Mutagenicity.

(b) Mutagenicity

In 1978, Dilley et al [443] demonstrated that 2,4,6-TNT was mutagenic in the Salmonella assay. Only single-plate values for a representative assay were reported. Doses of 10, 50, 100, 500, 1,000 and 5,000 (u)g of 2,4,6-TNT per plate were tested in TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with or without metabolic activation. Without metabolic activation, the 2,4,6-TNT induced a positive mutagenic response at 500 (u)g/plate in TA 1538, TA 98, and TA 100. Results at 100 (u)g/plate were ambivalent. At levels of 1,000 and 5,000 (u)g/plate, TNT proved to be cytotoxic. Addition of the S-9 activation mixture resulted in a reduction of both mutagenic response and cytotoxicity. Thus, 2,4,6-TNT induced frame-shift mutations in the Salmonella assay, with and without metabolic activation. However, the addition of microsomal activation mixture reduced the mutagenic response. This decrease is probably attributable to increased oxidative metabolism, thereby reducing the formation of cytotoxic and reactive reductive metabolites.

In a 1976 study by Won et al [146], TNT (explosive grade, primarily 2,4,6-TNT) also induced a positive mutagenic response in the Salmonella assay without metabolic activation. TNT induced a linear, dose-dependent mutagenic response in TA 98 at concentrations of 0.5-10 (u)g/liter in the agar overlay,

without metabolic activation. At concentrations exceeding 10 (u)g/liter, TNT was cytotoxic in TA 98, and no revertants were observed. After addition of the microsomal activation system, TNT did not elicit a mutagenic response in TA 98. Thus, TNT again induced frame-shift mutations.

The TNT metabolites, 2,6-dinitro-4-hydroxylaminotoluene, 4-amino-2,6-dinitrotoluene, 4,6-diamino-2-nitrotoluene, 2,4-dinitro-6-hydroxylaminotoluene, and 6-amino-2,4-dinitrotoluene, were also tested by Won et al [146] in the Salmonella assay. No data were presented for these experiments. However, the authors did note that revertant counts were essentially similar to those of controls. No mention was made of microsomal activation. The significance of these findings cannot be ascertained in the absence of data on concentration, range used, cytotoxicity, and microsomal activation.

In a 1978 cytogenetics study by Dilley et al [443], Sprague-Dawley rats were fed 0.002% and 0.25% 2,4,6-TNT in the diet for 28 days. No chromosomal aberrations were observed in bone marrow cells in any of the rats. Fifty metaphases per animal were counted. In contrast, a 1974 cytogenetics study by Geshev and Kincheva [682] did report increased chromosomal aberrations following TNT exposure. A 30% solution of TNT was applied to the skin on the back of 10 rats, 5 times a week for 6 months. Half of these rats exhibited structural changes in bone marrow: chromatid gaps, chromosomal gaps, breaks, and dislocated fragments. An average of 30 mitoses per rat were counted, and 20% of the mitoses contained structural changes. None of the control rats showed any chromosomal aberrations. The authors considered the positive cytogenetic toxicity to be a possibly important indicator of teratogenicity; however, such an attribution appears to be unsubstantiated by current scientific theory.

(c) Reproductive Effects

In 1973, Geshev [166] reported the results of a study of women who worked directly with TNT. The investigators were interested in effects on the reproductive system and their relationship to length of employment. A total of 181 women were studied, including groups of machinists and packer who were exposed to TNT and a control group with similar working conditions but no TNT exposure. The machinists and packers were exposed to TNT absorption via the respiratory tract and the skin.

Inflammation of the cervix, vagina, uterus, ovaries, and parametrium, as well as changes in the position of the reproductive organs due to unspecified pressure, were found in 67.1% of the machinists and in 58.5% of the packers, compared with 47.1% of the controls. Geshev [166] found that these effects increased with an increase in TNT exposure time. The difference in incidence between machinist and control groups was significant ($P < 0.05$), as was the difference between both machinist and packer groups and the control group ($P < 0.05$). These effects increased from 46.6% among machinists with less than 2 years of service to 77.1% among those with more than 5 years of service, an increase of more than 66%. The author did not quantify TNT exposure, present the demographics of the exposed and control groups, discuss the marital status or obstetric history of the subjects, nor list the diagnostic criteria applied. Therefore, it is not possible to state that the observed effects were due to TNT exposure.

Correlation of Exposure and Effects

(a) Systemic Effects

(1) Aplastic Anemia and Other Effects on the Blood

Aplastic anemia has been reported in humans following occupational exposure to TNT. It is a bone marrow disorder characterized by a reduction in hematopoietic tissue, fatty bone marrow replacement, and pancytopenia [810]. Active bone marrow can still be present, usually in small pockets surrounded by fatty tissue. The pathogenesis of aplastic anemia has not yet been elucidated, but investigators suspect a defect in either the multipotential bone marrow stemcells or the bone marrow microenvironment. Evidence is contradictory as to which of the two is more important. The presence of "hot spots," or foci of hyperactive bone marrow, suggests a defect in the microenvironment, because repopulation of the bone marrow by these foci would otherwise occur. However, the success of bone marrow transplantation indicates that the microenvironment is adequate. This suggests that all remaining stemcells in aplastic bone marrow are abnormal, as evidenced by the presence of abnormal maturing erythroid cells.

Thirteen fatal cases of aplastic anemia were reported in US munition plants from June 1, 1941, to September 1, 1945 [65]. The average age of the victims was 45 years, following a median exposure of 216 days. Exposure levels were not reported, although it was stated that only 33% of the 22 fatalities in that period involved exposure to TNT concentrations in excess of 1.5 mg/m^3 . Eight similar cases, seven fatal, were reported in workers in bomb-loading facilities [5,7,75]. Worker exposure was 0.5 mg/m^3 for 30 months for the one female survivor. Exposure for the seven male workers who died ranged from $1.1\text{-}7.0 \text{ mg/m}^3$ for 1.5 months to $2.1\text{-}3.5 \text{ mg/m}^3$ for 24 months and $3.7\text{-}10.7 \text{ mg/m}^3$ for 11 months.

Of the 24 cases of aplastic anemia attributed to occupational exposure to TNT during WWII in the UK, 15 were fatal [471]. The author noted that early effects of TNT on the blood, such as hemolysis and methemoglobinemia, could be compensated by hyperactive bone marrow. With progressive exposure, failure of the bone marrow occurred and resulted in aplastic anemia. Cyanosis, probably due to methemoglobinemia, was observed in only 6 of 24 cases.

Exposure of 64 workers to TNT concentrations ranging from 0.3 to 1.3 mg/m³ for 4-11 weeks (8 hours/day, 5-6 days/week) resulted in decreased levels of Hb and erythrocytes (red blood cells), indicating anemia, and in increased reticulocyte count, indicating compensatory increased red blood cell formation [606]. Exposure occurred by dermal and po routes, as well as by inhalation; thus, ambient TNT levels may not be a precise measure of total TNT exposure.

Exposure of 38 workers to TNT levels of 0.1-1.2 mg/m³ for an average of 9.9 years resulted in a decreased Hb level, compared to that of a matched control group of 20 [8]. Intermittent exposure to TNT levels from 2 to 10 mg/m³ for 5.5 months resulted in only transitory initial leukocytosis, accompanied by moderate eosinophilia [523,608]. All the workers had previously been exposed to TNT for at least 6 months, and most of them had been exposed for over a year.

No hematologic effects were observed in a controlled study on 45 workers exposed for 5 months to levels of TNT between 0.3 and 0.8 mg/m³ [81,264]. However, liver effects were observed. A study started in 1975 of 626 workers exposed to TNT and RDX in US ammunition plants resulted in the identification of a dose-response curve for decreased Hb levels due to TNT exposure [494]. The proportion of people with abnormally low Hb concentrations was greater in the exposed group than in the control group, even at TNT concentrations

as low as 0.10-0.19 mg/m³. However, the analytical method tested for TNT dust but not airborne vapors. Skin and oral exposure to TNT were not taken into account. Nevertheless, the findings appear to indicate that adverse hematologic effects follow exposure to TNT dust at concentrations of 0.10-0.19 mg/m³.

Animal studies with TNT showed methemoglobinemia with resultant cyanosis to be one of the major acute effects of TNT exposure. Cats administered ip doses of 20-80 mg/kg TNT had a minimum of 33% methemoglobinemia [88]. Formation of Heinz bodies was also induced by TNT exposure [88]. Transient cyanosis, probably due to methemoglobinemia, was observed after administration of about 1 g/kg TNT by gavage to rats in LD₅₀ studies [441].

(2) Liver Effects

Several signs of liver damage have been found in persons exposed to TNT: toxic jaundice or hepatitis, necrosis, and elevated levels of serum enzymes such as LDH and SGOT. Eight fatal cases of toxic hepatitis due to TNT exposure occurred in US munition plants between June 1, 1941, and September 1, 1945 [65]. Subjects had much shorter exposure and lower average age than those of the workers who died of aplastic anemia, discussed previously. The significance of these differences is unknown, but they appear again in later studies.

Necrosis of the liver was observed in one female and two male workers exposed for 6 months to TNT po, dermally, or by inhalation [474]. The exposures were not detailed. Necrosis was indicated by the intensity and duration of the jaundice in these cases of toxic hepatitis, as well as by decreased levels of blood cholesterol and prothrombin, and by increased urinary excretion of hippuric acid and galactose.

Functional liver impairment was observed as increased levels of bilirubin and B-lipoproteins in the sera of 167 workers in a TNT production plant [152]. No exposure levels were given. Of the total, 80% of the workers were women, 40% of whom were exposed for at least 6 years and 60% of whom were exposed for more than 10 years. A study of 106 persons exposed to TNT concentrations averaging 1.4 mg/m^3 for an unspecified period of time resulted in enlarged livers in 15% of the men and 9% of the women [465]. Liver dysfunction, as measured by the Quick test, was observed in 36.4% of the men and 49.8% of the women.

Statistically significant increases in serum levels of LDH and SGOT, indicating liver damage, were observed in workers exposed to 0.8 mg/m^3 TNT, compared with those of workers exposed to 0.3 mg/m^3 [81,266]. Increased values were usually seen when ambient levels of TNT exceeded 0.6 mg/m^3 . Cessation of exposure resulted in a decrease in serum enzyme activities of LDH and SGOT, but the time to baseline was not established. The maximum exposure period was 5 months.

In animal studies, TNT exposure also resulted in liver effects. Dosing of rats by gavage of sc with TNT in excess of 400 mg/kg resulted in porphyrinuria, excess excretion of porphyrin in the urine, indicating liver dysfunction [503]. Liver damage of an unspecified nature was also observed.

In a study with Rhesus monkeys, two of six animals receiving 10.0 mg/kg TNT daily for 13 weeks by gavage developed hemosiderin deposits in the liver cord cells [243]. In rats, administration of a diet containing 50% fat and 150 mg/kg TNT for up to 6 weeks resulted in fatty infiltration and necrosis of the liver [486]. Cats and rabbits administered high doses of TNT developed interlobular round-cell, as well as fatty infiltration of the liver [484].

Administration of TNT to dogs, at unspecified doses, resulted in degeneration of the liver and congestion of efferent capillaries [491].

(3) Effects on the Eye

Excess incidence of cataracts in exposed TNT workers has been reported [150,422,469,604]. All of these studies were performed in Eastern Europe. However, none of the experiments used proper controls, thus making the association between TNT exposure and cataract development questionable. Administration of a paste of 50% TNT in peanut oil to the eyes of rabbits did not result in any eye irritation at 24 or 72 hours after treatment [441].

(4) Skin Effects

Dermatitis and skin rashes have been reported in workers exposed to TNT [598,606,622]. Excessive perspiration, further irritation, and oily skin tended to aggravate these conditions. In animal tests, application of a 50% TNT paste in peanut oil on the intact or abraded skin of rabbits resulted in mild irritation [441].

(5) Neurologic Effects

Neurologic effects were observed in humans exposed to TNT concentrations slightly in excess of 1 mg/m^3 for 6-25 years [604]. Effects included unstable pulse, arterial pressures, headaches, dizziness, irritability, poor memory, and sleep disturbances. However, due to the lack of a control group, no positive association between TNT exposure and neurologic effects could be made.

Neurotoxic effects were observed in animals exposed to TNT. Convulsions were observed in rats and mice dosed by gavage with about 1 g/kg TNT [441]. Myocardial depression and hypothermia were observed in guinea pigs and rabbits

treated with unknown doses of TNT [506]. Cats given 40-100 mg/kg ip exhibited paralysis of the rear limbs, hypothermia, and narrowed pupils [88].

(b) Carcinogenic, Mutagenic, and Reproductive Effects

No studies on the carcinogenic effects of TNT were found. However, TNT was shown to cause frame-shift mutations in two Salmonella assays without metabolic activation [146,443] and in one assay with metabolic activation [443]. TNT was also shown to be cytotoxic in both Salmonella assays [146,443]. Two cytogenetic studies of chromosomal bone marrow abnormalities following TNT exposure of rats were reported [443,682]. In one study, no abnormalities were observed following exposure of rats to 0.002% or 0.25% TNT in the diet for 28 days [443]. Another study [682], however, reported chromosomal abnormalities in half the rats exposed to a 30% solution of TNT applied to the skin 5 days a week for 6 months. Structural changes were found in 20% of the metaphases. Thus, TNT appears to elicit a mutagenic response in two different systems. Taking into consideration the possible carcinogenic nature of the hydroxylamino intermediates observed during TNT metabolism in humans [623], it becomes apparent that caution should be observed in the use of TNT.

One study examined the human reproductive effects of TNT [166]. The experimental design was not strict enough to allow a correlation of TNT exposure with reproductive effects.

(c) Summary

The two main effects of human TNT exposure were aplastic anemia and toxic jaundice or hepatitis. Other blood abnormalities, such as methemoglobinemia and anemia, were also observed. Decreased hemoglobin concentrations occurred following TNT exposure to levels as low as 0.10-0.19 mg/m³.

Indications of low grade liver damage were also observed following low-level TNT exposure. Such damage is probably degenerative and probably results from the storage and metabolism of TNT in the liver. During this metabolism several reactive intermediates are formed, possibly resulting in cellular damage. Neurotoxic, skin, and eye effects were also observed. However, no positive association between these effects and TNT exposure could be made.

TNT was shown to be mutagenic in two reported Salmonella assays and in one of two cytogenic studies reported. Thus, TNT appears to cause molecular changes in DNA, indicating possible carcinogenic activity.

III. EVALUATION AND CONTROL OF EXPOSURE

Environmental Sampling and Analytical Methods

(a) Sampling

Breathing zone and area sampling are critical to the industrial hygiene evaluation of employee exposures to trinitrotoluene (TNT). Results can indicate appropriate engineering controls and work practices to reduce exposure when necessary.

A number of air sampling techniques have been used by researchers who have specified methods of collecting TNT, although none of the reports have included sampling efficiencies. In one of the earlier studies, also described in the section on Environmental Concentrations, Goodwin [80] reported an extensive air sampling program conducted in 1952 at the Lone Star Army Ammunition Plant. Midget impingers containing diethylaminoethanol (DEAE) were used to collect air samples contaminated with TNT. The DEAE in the impingers dissolved the TNT from the air so that the concentration of TNT could be analyzed. Midget impingers containing benzene were used by Friedlander and coworkers in 1974 [605], in addition to open-face, glass fiber (type A) filters. Type A filters were also used by Buck and coworkers [494] in 1975. These researchers collected breathing zone air samples with 37-mm filters on battery-operated MSA Model G or Bendix Model C115 belt-mounted pumps, operating within a flow range of 1.5-2.5 liters/minute. In a 1974 study with a 1976 followup, Morton and coworkers [81,264] collected TNT dust using Mine Safety Appliance Model G pumps. Filters were not mentioned.

Because TNT poses fire and explosive hazards [698,699], safety should be considered when selecting sampling methods for monitoring exposure levels. Early battery-operated sampling pumps were considered to be potential detonation hazards because of the electrical wiring and exposed metal surfaces. However, explosion-proof pumps are now available for use in explosive atmospheres. The US Departments of Interior and Health, Education, and Welfare issued joint regulations for certification of personal sampler units used in evaluating exposure to airborne coal mine dust, a potential explosive. These regulations were incorporated into the Code of Federal Regulations as Title 30 CFR Part 74 under the authority of the Federal Coal Mine Health and Safety Act of 1969 [544].

The original NIOSH-recommended sampling procedure for TNT [717] involved drawing a known volume of air through a glass fiber filter. This procedure did not capture TNT vapor, so NIOSH now recommends the addition of a midjet bubbler containing 15 ml of methanol in series with the filter to trap the vapor. The efficiency of the sampling procedure with the bubbler is not known, but it should be substantially better than the method without the bubbler, particularly at low concentrations of TNT. This method is discussed in detail in Appendix I.

(b) Analysis

TNT has been detected, identified, and quantified by a variety of analytical methods, including gas chromatography (GC), high-pressure liquid chromatography (HPLC), and plasma chromatography (PC), as well as spectrophotometry and mass spectrometry. PC appears to be the best routine method for analyzing trace amounts of TNT because it is sufficiently sensitive and

selective, operates at atmospheric pressure, responds rapidly, and is simple to operate [162]. Commercially-available, automated, TNT vapor detectors are very sensitive and useful when immediate analytical results are needed. However, they are subject to interference from other compounds and usually are not portable.

(1) Automated Instruments

In 1973, Harrison [374] presented a three-part collection of 14 reports on the evaluations of trace gas sampling and analysis by instrumental methods. From that collection, the most useful data for this document were drawn from reports II-10, 11, and 12, which evaluated actual performances of existing instruments. Automated instruments generally give high readings when interfering substances are present, but such high readings may be interpretable.

Report II-10 evaluated the performance of an automated GC analyzer. The unit used a platinum wire to adsorb and preconcentrate o-nitrotoluene, 2,4-dinitrotoluene (2,4-DNT), and 2,4,6-TNT vapors. After a collection period, the vapors were flash desorbed by heating the wire; they were then transported by the carrier gas to a 24-cm x 1.3-mm column of 15% diethylene glycol succinate (DEGS) on 30-60 mesh Chromosorb WAW (or T) and analyzed at 180 C. The separated effluent vapors were monitored with an electron-capture detector in direct current (dc) or pulsed modes. The unit's limit of detection for TNT was 1 ng; no limits were given for the other nitrotoluenes. Problems were encountered from contamination of the systems by high concentrations of DNT and TNT, causing significant "memory" of previous samples. High concentrations of other materials can smear out or interfere with DNT or TNT peaks

due to similar retention times. Therefore, this system may not work for the automatic monitoring of workroom conditions where contaminant concentrations are much higher than the parts per billion (ppb) level [374].

Report II-11 evaluated the performance of a PC. The chromatograph and the calibration device were placed in a controlled oven at 180-185 C. Air was used as the carrier and drift gases, and both positive and negative ion modes were used. The instrument had a reactant ion current of 0.4-0.45 nA, about .5 the normal value, because of a new grid design with higher wire density. Harrison found that peak identification by mass assignment was uncertain, because water vapor attaches to the "bare" ions and increases the observed molecular weight. He also found that the ion spectra were sensitive to the solvent; methanol produced a significant shift in the negative ion spectra but not in the positive ion spectra. The minimum detectable TNT concentration was 1 part in 10^{10} (mole fraction). A similar detection limit was found for DNT. No evaluations were made to test the PC's ability to distinguish between TNT and various isomers of DNT [374].

Report II-12 presented an evaluation of a quadrupole mass filter that ionized at atmospheric pressure. The unit was developed for biologic materials and was tested with 2,4,6-TNT and 2,4-DNT. Samples could be introduced as solids or liquids, or the injection port could be opened to draw in the atmosphere. The sample was ionized by radiation from a Nickel 63 source and then passed through an aperture into the quadrupole mass spectrometer. The response for TNT, per unit mass, with a 50-pg sample was 5.8 times higher than that for DNT. The limit of detection was about 1 part in 10^{10} (mole fraction) in the gas stream. While the unit worked satisfactorily for this analysis and separation, it is not a field unit and is not readily available [374].

Jurinski and coworkers [156] evaluated an automated device for colorimetric analysis and compared it with their GLC method. The colorimetric unit used by Hess and coworkers [175] employed a 15% solution of potassium hydroxide to form a colored Meisenheimer complex with any trinitroaromatic substance in the water. Color intensity was read at 440 nm. The unit detected 2,4,6-TNT to 1 ppm but tended to read high at this level due to color carryover in the automatic sampler. The results were reproducible and the unit was reliable, but interference from other trinitroaromatic explosives, such as 2,4,6-trinitrophenylmethylnitramine (tetryl), was a problem. The authors concluded that the relatively nonspecific automated method was useful for routine surveillance, but their GLC method was preferable when exact information on the quantities of specific compounds was needed and the extra time required for the analysis was justified.

Membrane separators have increasingly been used in trace-vapor detection technology to enrich organic compounds prior to their introduction into detection instruments. In 1975, Spangler [332] evaluated membrane performance for separating TNT vapor from carrier gas in two commercial "explosive" detectors: a quadrupole mass analyzer with a double membrane separator and a pulsed coaxial electron-capture detector with a single membrane separator. The two instruments had detection limits of 1 part in 10^{10} (mole fraction) and 2 parts in 10^{10} (mole fraction), respectively. The device with the double membrane separator was clearly more selective and less susceptible to interferences. Both instruments appeared to be designed for fixed installations, although there was no specific discussion of portability or sample line losses.

Karasek [162] reviewed the techniques available for developing automated instruments to detect TNT vapors in air: GC with electron-capture detectors,

mass spectrometry, combined GC and mass spectrometry, and PC. He found that GC instruments reportedly had low resolution, peak-tailing interferences, and significant memory problems because TNT was incompletely desorbed from sample concentrator surfaces. The mass spectrometry techniques were satisfactory for detecting and quantifying both TNT and 2,4-DNT, but had problems maintaining the required high vacuum for long periods. In addition, water and oxygen adversely affected the ion-generating filament of the mass spectrometer by decreasing its stability and causing insulating deposits to build up on its surface. Karasek concluded that PC offered the best approach to routine monitoring of trace amounts of TNT and DNT, because it was sufficiently sensitive, operated at atmospheric pressure, responded rapidly, and was selective and simple to operate.

Dynamic calibration of highly sensitive detectors for vapors from explosives is a problem because of the technical difficulty in generating standards of <1 ppb. In 1976, Pella [260] reported having generated known concentrations of vapors of 2,4-DNT, 2,6-DNT, and 2,4,6-TNT as low as 0.05 ppb by passing nitrogen through thermostatically controlled 1.9-m x 6.4-mm columns containing a stationary phase of 11% explosive on 60-80 mesh Chromosorb G. The saturated vapors were diluted to the desired concentration by a single-stage diluter, which used differential flow controllers on the streams of vapor and air to achieve dilutions up to 200 times. Dilutions above 1,000 times were obtained by producing very low flows of vapor with an electronic metering valve. The systematic error for TNT concentrations was 15-20%. Calibration of the system was performed with 1-mg charcoal tubes that captured 100% of the TNT vapors at flowrates up to 410 ml/minute. Satisfactory calibrations were

obtained on three unnamed vapor detectors, all of which used electron capture. This system may also be of use for calibrating other sampling and analytical equipment.

(2) Chromatographic Methods

Gas-liquid chromatography (GLC) is an accurate and precise method for analyzing trace quantities of nitrotoluenes. In 1972, Alley and Dykes [82] described a detailed, well-conducted laboratory study of estimation by GLC of 24 nitrate esters, stabilizers, and plasticizers, including 2,6-DNT, 2,4-DNT, and 2,4,6-TNT in nitrocellulose-base propellants. Stainless steel columns of 61 cm x 3.2 mm packed with a range of liquid phases and solid supports were evaluated: 3.8% OV-101 on Gas Chrom Q, 2.5% OV-210 on Chromosorb W-HP, and 1.1% OV-225 on Gas Chrom Q. Temperature programming was used in conjunction with a flame-ionization detector. The best separations of the three nitrotoluene compounds were achieved with the OV-225 column. Good-quality chromatograms were produced, and the relative standard deviation was <1% for replicate extractions and analyses of a number of compounds, not including the three nitrotoluenes. The method appeared highly sensitive, although detection limits were not determined.

In 1977, Leggett [216] studied the vapor pressure of 2,4,6-TNT by GLC and developed a sensitive, useful method for TNT analysis. TNT was separated from other volatile compounds on a 1.8-m stainless steel column packed with 10% Dexsil 300 GC on 100-120 mesh Anakrom ABS, which was operated isothermally at 190 C with a tritium-foil, electron-capture detector. The detector response was linear over the range of 0.01-0.1 ng. Leggett reported that other isomers of TNT and those of DNT can also be separated and quantified by this approach.

Legget [216] also evaluated problems of vapor sample injection and found that 2,4,6-TNT vapor was efficiently adsorbed on the needle of the gas-tight syringe. Reproducible injections were obtained only if the injection port temperature was set high enough (250 C) to vaporize TNT in the needle, and only if the outside of the needle was wiped off with a tissue slightly dampened with acetone before injection. This wiping procedure is important for all methods using direct injection of airborne vapor samples.

Jurinski and associates [156] developed a GLC method to separate and quantify DNT and TNT isomers, and then compared the method with an automated colorimetric method. The authors used a 3.3-m x 3.2-mm stainless steel column of 10% UC-W 98 silicone gum rubber on 80-100 mesh Chromosorb O, which was operated isothermally for 4 minutes at 130 C and then programmed up to 210 C at 8.5 C/minute. All mononitrotoluene (MNT) isomers were eluted as a single peak and then followed in order: 2,6-, 2,5-, 2,4-, and 3,4-DNT and 2,4,6-, 2,4,5-, and 2,3,4-TNT. The 2,3- and 3,5-DNT isomers were not used but, based on the data developed by Parsons and associates [119] in 1961, these two would probably overlap with the 2,5- and 3,4-DNT peaks, respectively. The nitrotoluenes were extracted from water samples with benzene, which efficiently recovered 97% of 2,4,6-TNT with a single extraction. The GLC analysis, using a flame ionization detector, readily detected 2,4,6-TNT at 1 ppm in 100 ml of water. Sensitivities for other isomers of TNT and for those of DNT were not determined; however, the flame ionization detector is likely to be equally sensitive for determining these isomers. No decomposition of the test compounds was observed.

In another study, Juhasz et al [340] developed an HPLC micropreparative separation technique for p-nitrotoluene, 2,4-DNT, and 2,4,6-TNT, which allowed

determination of collected sample fractions by infrared (IR) radiation and mass spectra. This technique can be used in the verification of chromatogram peak identification. However, the authors were not successful in trapping the MNT compound because of its volatility. A 1-m x 3-mm column of Corasil II, microparticles of silica gel layered over a solid spherical core, was used with a solvent system of 5% chloroform with cyclohexane. The nitrotoluenes in the column effluent were detected with an ultraviolet (UV) detector set at 254 nm. This column was chosen because it gave high quality peaks and separations using 30-40 (u)g samples. Other columns, coated packings, silica gels, chemically bonded stationary phases, and spherical, porous-layered beads with low, specific surface areas were rejected because of potential interference with spectral measurements, poor quality chromatograms, or low-sample capacities. Good quality IR and mass spectra were obtained for 2,4-DNT and 2,4,6-TNT. No tests were run with other isomers of MNT, DNT, or TNT.

In another HPLC method, Stanford [248], in 1977, separated 2,4,6-TNT and four of the six DNT isomers: 2,4- and 3,4-DNT were not resolved. A 25-x4.6-cm partial 10-ODS column was used with a mobile phase of 40% methanol and 60% water. The nitrotoluenes in the column effluent were detected with a UV detector set at 230 nm. The minimum detectable amounts for DNT and TNT in this study ranged between 5 and 25 ng.

The most directly applicable method for sampling and analyzing TNT is a variation of NIOSH Method No. S226 [717]. In the original NIOSH method, TNT was collected on a glass-fiber filter. However, this procedure fails to collect the vapors of TNT and, thereby, underestimates the airborne concentration of TNT. It has been shown [717] that TNT particles and vapor can be

collected effectively by using a glass fiber filter in series with a midget bubbler containing 15 ml of methanol. After sampling, the filter is added to the bubbler, which is sealed and shaken to desorb the collected TNT. Methanol should be added until the volume reaches the 15-ml mark. An aliquot of the sample is then analyzed by HPLC on a 300-x 3-mm column of (u)-Bondapak C-18 with a 65:35 methanol to water solvent elution system. Isomers of TNT are recognized by elution times and estimated by UV absorbance at 254 nm. This method is described in more detail in Appendix I.

Environmental Concentrations

The previously described sampling and analytical methods have been used to determine TNT concentrations in the workplace. Unfortunately, some of the studies measuring environmental levels of TNT do not describe the methods used or the number of samples collected [7,80,118,135,150].

In 1972, Goodwin [80] reported an extensive air sampling program conducted in 1952 at the Lone Star Army Ammunition Plant in Texarkana, Texas. The purpose of the study was to assess the need for installing equipment to reduce exposure to trinitrotoluene (TNT). Airborne TNT was sampled by drawing air through midget impingers containing DEAE. The results of the study are presented in Table III-1.

In 1974, El Ghawabi et al [8] reported data on environmental levels found in an Egyptian TNT-manufacturing and shell-loading plant. Samples of air collected from most areas of the plant had mean TNT concentrations of <1.0 mg/m³. However, concentrations as high as 10 mg/m³ were reported at the crushing area, where exposures were intermittent and where the operation was not performed on a daily basis. The environmental concentrations of TNT in the plant are presented in Table III-2.

TABLE III-1

CONCENTRATIONS OF TNT IN AREA AIR SAMPLES AT THE

LONE STAR ARMY AMMUNITION PLANT*

FEBRUARY 4-8, 1952

Operation	Contaminant Form	Amount Found (mg/m ³)	Remarks
Mixing and feathering TNT	Fume	1.4	Dopp kettle no. 3
	"	0.8	"
Melting TNT	"	0.9	Melt unit no. 3
	"	2.9	"
Screening TNT	Dust	1.3	Filling hopper of screen
	"	0.2	"
	"	0.5	Emptying screen
	"	0.7	"
Assembling grenades	"	0.5	Placing covers on grenades
	"	9.5	Wiping grenades
	"	2.6	Placing covers on grenades
	"	9.5	Wiping grenades
	"	2.4	Placing covers on grenades
Pouring TNT	Fume	3.1	Multiple pour control
	"	0.9	Handling skids of shell
	"	0.5	"
Pellet Insertion	Dust	1.9	"
	"	1.7	"
	"	1.6	"
	"	4.7	"
Pouring TNT	Fume	2.4	Multiple pour control
Mean		2.34	

*Formerly Lone Star Ordnance Plant

Adapted from reference 80

TABLE III-2

INT CONCENTRATIONS IN AIR AT DIFFERENT WORKPLACES

IN AN EGYPTIAN INT PLANT

Site	---During Summer---		---During Winter---	
	Range	Mean	Range	Mean
	(mg/m ³)		(mg/m ³)	
At nitration				
Subdivision 4	0.15-0.65	0.25	0.1-0.4	0.2
Subdivision 5	0.8-1.20	1.00	0.6-0.9	0.8
At crystallization	0.25-0.70	0.50	0.3-0.6	0.4
At filter	0.40-0.90	0.75	0.4-0.7	0.6
At washing	0.25-0.75	0.50	0.2-0.5	0.3
At crushing	6-10	7.5	2-5	3
At the distillation unit	0.4-0.6	0.5	0.1-0.3	0.2

Adapted from reference 8.

Environmental concentrations of TNT dust in Ukrainian iron ore mines during the loading of bulk explosives into drill holes have also been reported [150]. The following TNT concentrations were measured in the workers' breathing zones: 0.65 ± 0.12 mg/m³ for workers filling flooded holes with granulite containing 21% TNT in the form of scales, and 12.51 ± 3.31 mg/m³ for those filling dry drill holes with the granulite.

Hassman and Hassmanova [135] reported environmental concentrations of TNT in a munitions plant and in a manufacturing plant for industrial-explosives using a mixture of 19% TNT and 81% ammonium nitrate. The results of the study are presented in Table III-3. In the munitions plant, where 10 samples were taken, the mean concentrations of TNT ranged from 0.045 mg/m³ in the boiler filling operation to 0.76 mg/m³ in filling with lumps and then casting. In the second plant, where 15 samples were collected, the mean concentration of TNT ranged from 0.31 mg/m³ in packing to 0.93 mg/m³ in filling small cartridges. Unfortunately, these exposure data cannot be compared, because the collection times and sampling periods were different for each plant. In the first plant, measurements were taken between 10 am and 1 pm, with a 20-30 minute sampling period for each sample. In the second plant, measurements were taken between 8 am and 1 pm, with a 3-5 hour sampling period for each sample.

In 1973, Morton and coworkers [81,264] conducted a study of the biologic effects of TNT exposure after a routine industrial hygiene survey indicated that atmospheric levels of TNT were above the current threshold limit value (TLV) of 1.5 mg/m³. During the 5-month study, which is discussed in Chapter II, 8-hour time-weighted average (TWA) concentrations ranging from 0.3 to 0.8 mg/m³ were reported.

TABLE III-3

ENVIRONMENTAL CONCENTRATIONS OF TNT IN MUNITIONS PLANTS

Plant	Procedure	Number of Samples	TNT in Air (mg/m ³)	
			Variance	Mean
A	Filling the boiler	2	0.04-0.05	0.045
	Pouring	2	0.31-0.14	0.32
	Filling with lumps and casting	1	0.31-1.45	0.76
B	Cleaning and packing the product	2	0.18-0.20	0.19
	Filling of large cartridges	3	0.19-0.42	0.32
	Filling of small cartridges	3	0.43-1.69	0.93
	Packing	9	0.09-0.56	0.31

Adapted from reference 135

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In 1944, Eddy [7] described three cases of aplastic anemia in workers employed at bomb line operations, in the shipping room and cooling bay or in maintenance. This investigator reported TNT concentrations of 1-3.5 mg/m³ in a cooling and puddling room and concentrations below detectable levels in a shipping room. In another report describing four cases of aplastic anemia among melt unit operators, Hart and coworkers [5] cited concentrations of TNT ranging from 1.1 to 10.7 mg/m³ in work areas at a bomb loading plant. Concentrations measured in each case are included in Table III-4, which provides a summary of the literature on environmental exposures for TNT.

Engineering Controls

Engineering design for controlling TNT in the workplace should maintain air concentrations well below the recommended limits, minimize skin contact, and safeguard against fire and explosion hazards.

In manufacturing plants, closed systems, properly maintained and operated, should be used to reduce the possibility of TNT vapors or aerosols escaping into the workroom air and to minimize the likelihood of skin contact. Where closed systems are not feasible, well-designed local exhaust ventilation should be provided. However, both of these systems may still allow TNT emissions through openings for adding, testing, or removing the contents, or through leaks in the system. To prevent emissions from leaks, all equipment should be inspected frequently and repaired promptly when problems develop. Special attention should be given to the conditions of seals, joints, access ports, and similar points of probable wear or damage; these areas should be inspected regularly. They should also be designed to resist deterioration.

TABLE III-4

SUMMARY OF TNT ENVIRONMENTAL DATA

Location of Exposure	Concentration Range (mg/m ³)	Number of Individuals Exposed	Reference
Shipping room	Case 1: below detectable levels	1	7
Cooling room	Case 2: 1-1.5	1	7
Puddling room	Case 3: 1-3.5	1	7
Belt unit, during charging	3.7, 10.7	1	5
TNT pouring, tightening adapter plugs	4.2	1	5
Sweeping and cleaning in puddling and cooling bays	1.1-7	1	5
Puddling room	5.3-7	1	5
Bomb and shell-loading plant	Case 1: 1.1-4.2 Case 2: 2.1-3.1 Case 3: <0.5	1 1 1	74
Shell-loading ammunitions plant	0.2-9.5 (Mean=2.38)	21	80
Various working places within TNT-manufacturing plant	0.1-1.2 10, peak fluctuation (crushing area)	35	8

TABLE III-4 (CONTINUED)
SUMMARY OF TNT ENVIRONMENTAL DATA

Location of Exposure	Concentration Range (mg/m3)	Number of Individuals Exposed	Reference
Pit mines	0.65-12.51	3	150
Ammunitions plant and industrial explosives manufacturing plant	Plant A: 0.045-0.76 Plant B: 0.31-0.93	25	135 135
Ammunitions plant	0.3-0.8	5	81
Ammunitions production	6	-	675
TNT workers	0.5-2.0	17	523
Pill shop	0.3-0.6	52	606
Pill shop and melt house	0.3-1.3	10	606
Washout facilities	<0.03-4.00	24	605
Bagging operations	0.62-4.00	2	605
TNT workers	0.08-0.59	12	493
"	0.3-0.8	43	81
"	<0.01-1.84	33	494
"	1	574	604
Uncovered reactors	<0.01-0.34	-	152
Reactor sampling hatch	0.01-0.60	-	152
TNT cleaning equipment	0.01-1.33	-	152
Cleaning sampling hatch	0.01-2.00	-	152
Drying equipment	0.02-10.00	-	152
Weighing machines	0.06-40.00	-	152

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If local ventilation is used, the face of the exhaust enclosure should be minimized. This will reduce the volume of air required to control fugitive emissions. Airflow should be monitored weekly.

TNT is classified by the National Fire Protection Association (NFPA) as a highly explosive compound that can be detonated by strong shock or temperatures above 240 C [699]. To minimize the propagation of an explosion, facilities should be composed of detached units equipped with explosion-venting systems, such as blow-away walls or ceilings, and pressure-resistant walls. Whenever practical, each TNT-handling unit should be subdivided with fire- and pressure-resistant walls to restrict hazardous areas and to prevent direct communication with other areas [559,702,704].

Accidental fire and explosion can be reduced, or the force minimized, by removing TNT ignition sources. For example, in 1942, the Picatinny Arsenal [261] discovered in fume ducts above TNT melting units sublimed TNT, which is distinctly more sensitive than ordinary crystalline TNT. In 1970, Groothuizen et al [212] investigated the cause of an explosion in a TNT melting shop. The authors suggested that contaminants such as cardboard fibers decreased the thermal stability of TNT, and the instability initiated explosion.

Unusually high concentrations of TNT can also pose a fire or explosion hazard. Elevated concentrations of TNT vapor can occur above liquid surfaces when the substance is heated above its melting point of 82 C [26] and when vapors condense on containers or other surfaces [699]. These phenomena indicate that nonsparking and noncombustible construction materials should be used in ventilation systems. TNT dusts and vapors should be trapped before entering the outside environment by using dust and vapor collectors such as

wet cyclones and air impellers. These collectors should be located outdoors or in detached rooms with adequate explosion vents. Collectors should be cleaned regularly to prevent accumulation of trapped explosives. The principles to be applied in the control of atmospheric concentrations of TNT in the workplace are detailed in NIOSH's Recommended Industrial Ventilation Guidelines [539]; in Industrial Ventilation--A Manual of Recommended Practice [669], published by the American Conference of Governmental Industrial Hygienists (ACGIH); and in Fundamentals Governing the Design and Operation of Local Exhaust Systems, Z9.2-1971 [538], published by the American National Standards Institute (ANSI). Electrical wiring and equipment should conform to NFPA recommendations [701] for hazardous locations and should include explosion-proof fixtures and wiring where appropriate. NFPA also publishes Recommended Practice on Static Electricity, publication 77-1977 [703].

Work Practices - Control of Safety Hazards

Work practices that reduce safety hazards due to the explosive nature of TNT should be used in conjunction with or instead of engineering controls. Practices involved in the manufacture, use, transportation, and storage of explosives are regulated by government agencies to protect workers and the general public. Regulations of the Federal government include the following: Bureau of Alcohol, Tobacco, and Firearms, 27 CFR 178 and 181; Department of Labor, Occupational Safety and Health Administration (OSHA), 29 CFR 1910 and 1926; Environmental Protection Agency (EPA), 40 CFR 457 and 700; Office of Surface Mining Reclamation and Enforcement, 30 CFR 700-830; Mine Safety and Health Administration (MSHA), 30 CFR 15, 55-57, 77; Department of Transportation (DOT) 49 CFR 171-178; and Coast Guard, 49 CFR 176. Many other government agencies and private organizations have also

published guidelines for handling explosives. These include: Bureau of Mines (BOM) [667]; Institute of Makers of Explosives (IME) [537,578,579]; and the NFPA. The Department of Defense (DOD) has Ammunition and Explosives Safety Standards [560,642], and contractors must conform to standards in the DOD Contractors' Safety Manual for Ammunition, Explosives, and Related Dangerous Materials [549]. Transporters of explosive materials must conform to DOT regulations in 49 CFR Parts 171-178, and should use procedures described in these publications: Code for the Manufacture, Transportation, Storage, and Use of Explosive Materials, NFPA Publication No. 495-1973 [702]; Suggested Code of Regulations for the Manufacture, Transportation, Storage, Sale, Possession, and Use of Explosive Materials, IME Safety Library Publication No. 3 [583]; and Recommended Industry Safety Standards, IME Safety Library Publication No. 6 [584].

In the United States, TNT is commonly manufactured by adding mixed concentrated nitric and sulfuric acids to toluene in either continuous or batch enclosed processes. NIOSH's recommended standards for handling nitric [504] and sulfuric acids [541] should be followed when applicable.

The amount of TNT kept at a manufacturing or packaging site should be limited to the amount needed for a single shift. Similarly, finished explosive products should be removed as soon as possible to a specially-designed storage magazine. These practices minimize the danger of detonation.

Another practice that minimizes danger and possible loss of life is the establishment of controlled areas. Entry into areas of TNT-production and explosive manufacture should be restricted to authorized personnel who are trained to recognize the safety hazards. To reduce the possibility of accidental detonation, the number of persons working in these controlled areas

should be limited to those actually needed for the operation. Areas in which TNT is produced or used should be bounded by curbs, dykes, or walls to retain any spills. The air should be monitored at random times to ensure employee safety and health.

The amount of material and the number of people allowed within each facility should be posted outside the building. An entry log, also located outside the restricted area, provides a record in a safe place indicating whether or not the occupancy limit is being exceeded and who is or was in the area at some particular time. This log should be checked periodically by a designated employee. If an explosion does occur, the log can be used to determine who was in the area at the time.

Work practices should be designed to eliminate sources of ignition, prevent dust accumulation through proper housekeeping, and minimize the dangers of impact detonation. Open flames, smoking, and uncovered light bulbs should be prohibited in production areas. Motor-driven, portable equipment and internal combustion engines and vehicles, such as forklift tractors, should also be prohibited unless they are approved for use in an explosive atmosphere and are correctly maintained.

Maintenance and repair work inside the buildings should be prohibited while plant operations are in progress. Before such work is permitted inside a building, operations should be shut down, all equipment should be cleaned with hot water or steam, and the work area should be visibly inspected to ensure that there is no significant accumulation of TNT. A permit system should be established to ensure that these steps are taken. A responsible person, such as a shift supervisor, should sign each permit before maintenance or repair work begins. If maintenance and repair work is performed outside

the building, the above procedure may not be necessary. However, the shift supervisor or other responsible individual should make this determination. When maintenance is completed, the workplace should be inspected again to ensure that no hazardous materials such as spark-producing metals remain. Plant operations should not be resumed until the workplace is inspected and cleared by the responsible persons. All screws, bolts, and nuts on equipment must be thoroughly oiled before they are turned, to prevent spark production. A machine that has been shut down for a long period of time, or recently installed, should be operated with "dummy mixes" until it proves to be in satisfactory operating condition.

Because of the sensitivity of TNT to impact and detonation, precautions should be taken to avoid having loose objects in the work area. If flashlights are used for any type of work, they should be made of nonsparking material and equipped with wristbands to prevent impact and detonation. All special areas for smoking should be located far from regulated areas, and fixed lighters should be provided in these areas so that employees will not inadvertently carry matches or lighters to the work area. Any tools used in the presence of TNT should be sparkproof. Tools should not be left lying around in regulated areas.

Special precautions should be taken when work is to be performed in confined spaces or tanks that may contain, or have contained, nitrotoluenes; entry into these spaces should be controlled by a permit system. All lines to and from confined spaces should be disconnected or blanked off before entry, and the area should be emptied and cleaned with hot water, steam, or a specific solvent. If a solvent is used for cleaning, a water wash should follow, and the tanks should be purged with air. It is good practice to

continue blowing air through the tank at a low rate while an employee is working inside. In any case, the oxygen concentration within the tank should be checked before an employee enters and periodically while the tank is occupied. Portable, battery-operated oxygen monitors are available for this purpose [607]. Concentrations of TNT should also be monitored. An employee should enter the tank only after all these steps are taken and after a designated representative of the employer, such as the shift supervisor, signs the permit.

Personnel entering confined spaces should wear appropriate protective clothing and use either a self-contained, pressure-demand mode breathing apparatus or a combination supplied-air suit with an auxiliary self-contained air supply. Each person should also be equipped with a lifeline harness tended by another worker outside the space. This second person should be equipped for entry into the confined space with approved personal protective equipment and should maintain communication with the employee inside by sight, voice, signal line, telephone, radio, or other suitable means. A third employee, equipped to aid the other two if necessary, should provide general surveillance of their activities.

Engineering controls are the primary means for reducing worker exposure to TNT, but respirators may represent the only feasible and adequate source of protection during the installation or testing of a new exhaust system, during performance of nonroutine maintenance or repair, during single operations, or during emergencies.

At the end of each workshift, all equipment used in the TNT workplace should be cleaned. Clean cloths to be used for wiping equipment should be

kept in a covered container. Soiled or wet cloths should be discarded in covered waste containers kept outside the regulated areas.

All spills of TNT should be cleaned up immediately. Small spills can be wiped up by an employee who has been informed about the safe handling of TNT. Large-spill removal should be performed only by employees who are specially trained in the procedure and who are under the supervision of the safety department. The safety department should determine the cause of all large spills and institute corrective measures to ensure that similar spills will not occur again. TNT, a solid at room temperature but handled as a liquid at elevated temperatures, should first be allowed to cool and solidify before being cleaned up; then it should be shoveled into containers, using nonsparking equipment. The area of the spill should be washed with hot water or steam to remove any remaining TNT. The wastewater should be piped into holding tanks where the TNT can be either recovered for recycling or destroyed. Methods of waste disposal should comply with Federal, State, and local regulations.

Because TNT poses a potential hazard to safety and health, it is important for all employees to be trained in its proper handling. Instructions should be printed both in English and in the predominant language of workers who do not read English. Each new employee should be thoroughly informed of processes, potential hazards, necessary personal protective equipment to be used, and emergency procedures to be followed. Special care should be taken to inform employees of the hazards inherent in working with TNT and the precautions necessary to minimize exposure and injury from TNT compounds. Employees who

are unable to read printed instructions should be told about these hazards in a language they understand. In addition, all employees should be familiar with the common signs and symptoms of TNT intoxication; this information should also be made available to each worker's family in the event that the worker becomes ill off the job.

A thorough, on-the-job training program should be conducted for new employees, and safety meetings should be held regularly. Unannounced safety inspections should be made frequently, and continued instruction on safety should be given to workers to ensure caution. Particular attention should be given to employees who are unable to read labels, signs, and instructional materials so that they know and understand their work processes and the need for correct use of personal protective equipment and established emergency procedures.

In areas where TNT is handled, emergency entry and escape procedures should be prepared and made available in writing to all employees. Practice evacuation drills should be held periodically, and the emergency plan should be recapitulated at least yearly to maintain worker preparedness. Rapid evacuation is the single most important procedure for ensuring safety in an emergency. The employer is responsible for providing the necessary training to all emergency personnel.

Records should be kept of work practices, maintenance schedules, emergency procedures, storage locations, entry into magazines and other restricted areas, accidents, and employee exposures. Such records should be accessible to employees.

The location of magazines for storing TNT and materials containing TNT should conform to the requirements and recommendations of the appropriate

civilian or military authority. Magazines should be clean, dry, well ventilated, reasonably cool, and securely locked. Safety regulations governing the operation of each magazine should be posted on the inside of the magazine door. According to the IME [583], all magazines containing explosive materials should be opened and inspected at least every three days to determine whether or not there has been unauthorized entry. Doors should be locked except during inspections or while explosive materials are being placed in or removed from the magazines. Containers of explosive materials should be stored so that they can be counted and checked easily. Rigid containers should be laid flat, and cases should be stored with the top side up to minimize spilling if they accidentally break open.

Containers of explosive materials should be unpacked and repacked outside the magazine; only securely closed containers should be placed in the magazine. Tools used to open these containers should be constructed of nonsparking material. Magazine floors should be swept and washed regularly. When magazines need interior repairs, all explosive materials should be removed and the floors should be cleaned thoroughly both before and after repairs. When there is a possibility that sparks or fire may occur during repairs, all explosive materials should first be removed from the magazines.

Sealed, intact containers should be used for transporting and storing TNT, its raw material, and its wastes. A sealed container is one that prevents the release of TNT from the closing. An intact container is one that has not deteriorated or been damaged to the point where TNT is released from the container. These containers should be properly labeled as to contents and potential hazards. TNT in sealed, intact containers poses no threat of

exposure to employees; it should not be necessary to comply with monitoring and medical surveillance requirements in operations involving only these containers.

Work Practices--Control of Health Hazards

TNT is a potential health hazard in the industrial environment, having caused such adverse health effects in exposed workers as cyanosis [65], aplastic anemia [5,7,65,74,471], neurologic disturbances [72,598,606,622], inflammation of the reproductive tract [616], and liver necrosis [474]. Worker exposures to TNT can be minimized by effective engineering controls and by a program of work practices that reduces not only safety hazards, but also health hazards. The program should include the use of appropriate personal protective equipment and employee training.

The physical properties of TNT influence the type of worker exposure and, thus, the expected toxic effects. TNT has a melting point of 80.9 C and a vapor pressure of 1.82×10^{-4} mm Hg (2.43×10^{-2} Pa) at 50 C [61,250]. TNT is a solid at room temperature, so there is a potential for dust exposure. TNT is soluble in organic solvents [26], a property that may enhance its absorption through the skin. Direct skin contact with TNT can be reduced by protective clothing and good personal hygiene.

In 1971, Linch [712] discussed the permeability of a number of materials used in protective clothing. He said that cotton coated with butyl rubber was impervious to nitrobenzene, a nitroaromatic compound, and a Chem-Proof air suit made with this material had been used successfully by du Pont. However, he did not present evidence for these assertions. The same author [116,734] also concluded that no other commercially available elastomer approaches

butyl rubber for resistance to aromatic nitrocompounds. Again, however, he failed to present any data for these assertions.

JR McCarthy [verbal communication, February 1980] stated that his studies at du Pont showed butyl rubber to have varying degrees of permeability to aromatic nitrocompounds. Differences were attributed to formulations among manufacturers and thicknesses among rolls of the products. Therefore, McCarthy recommended that the permeability of each roll of butyl rubber be measured. Butyl rubber gloves have been shown by one company to be effective barriers to nitrotoluenes [370]. However, these gloves were found to crack easily and should be discarded every 2-3 days to prevent accidental skin contamination [570].

When mid-body contact with TNT is expected, butyl rubber aprons should be worn to prevent skin contamination. Butyl rubber safety shoes or leather safety shoes covered with butyl rubber overshoes are recommended for workers who handle TNT. The shoes should be thoroughly cleaned and ventilated after contamination; they should be discarded when tears, cracks, or signs of wear appear.

The pervious nature of work clothes to an aromatic nitro compound was discussed in 1975 by Marchenko and coworkers [152]. For TNT workers, they showed that sections of the skin covered by work clothes had TNT concentrations of 0.31 mg/dm^2 . Uncovered sections of skin contained 1.68 mg/dm^2 .

If a breakthrough of TNT occurs, the sensation of wetness can sometimes alert a worker, but some individuals may not feel this sensation so readily as others. Although TNT rapidly penetrates cotton, white cotton clothing was preferred by one plant [570] because contact caused a clearly visible stain. Another plant [570] used fire- and acid-retardant clothing, which does

not show stains but helps protect against burns that could occur during the manufacture of TNT. This plant also supplied workers with butyl rubber clothing when skin contamination appeared to be possible. Fire-retardant, disposable work coveralls and boots are commercially available [576], but their effectiveness in preventing skin contamination by TNT apparently has not been evaluated. In the event of skin contamination by TNT, contaminated clothing should be removed immediately and carefully to ensure that further skin contact does not occur. Contaminated skin areas should be cleaned immediately with soap and warm water to minimize absorption.

When the skin is contaminated with TNT, it is difficult to remove the compound completely [79]. In 1943, Norwood [79] reported that ordinary soap was inadequate for removing TNT from the skin as indicated by the Webster skin test. He suggested the use of a special liquid soap containing 5-10% potassium sulfite and 5-10% wetting agent. A brown-colored material is produced when TNT dissolves in the lather of this soap, so a worker could clearly see if skin contamination with TNT has occurred.

The difficulty of removing TNT from the skin with ordinary soap and warm water was also discussed in 1975 by Marchenko and coworkers [152]. They compared washings from uncovered sections of skin on the hands with those of sections covered by work clothes. The investigation quantitatively evaluated skin contamination, the protective effect of work clothes, and the effect of washing among workers exposed to TNT. The results are shown in Table III-5. These data indicate that showering removes a large amount but not all TNT from the skin. The amounts of TNT detected on the skin after working and after showering were statistically significant ($P < 0.01$). No significant difference ($P > 0.5$) was obtained in a comparison of the amounts

of TNT on the skin before working and after showering. Incomplete removal of TNT from the skin resulted in constant contamination, as indicated by the detection of TNT on the skin before work.

In 1974, Osina and Dymova [378] developed a method for the quantitative determination of TNT on skin and other surfaces, including fabrics. Cotton swabs containing ethanol were used to remove TNT from skin, and swabs containing acetone were used to remove TNT from other surfaces and fabrics. The swabs were then rinsed with ethanol and the resulting TNT solution was reacted with a solution of potassium hydroxide to give a red color. The intensity of the color was determined by spectrophotometry. The results, presented in Table III-6, showed that as many as three washings with ethanol soaked cotton were needed to totally remove TNT from skin. This method can be used to determine the presence of nitrotoluene on skin when contamination is suspected. It can also be useful in determining the effectiveness of showering to remove skin contamination.

In manufacturing areas or laboratories where solid or molten TNT is handled by workers, safety spectacles equipped with side shields should be worn for continuous eye protection. Chemical goggles should be used in all operations with potential for spills or splashes. Because TNT is solid at room temperature, the potential for dust exposure exists under this condition. Work clothes should not have cuffs, folds, or pockets in which dust might accumulate.

Work garments, including underclothing, should be cleaned after each work-shift. Workers should be prohibited from taking soiled outer or inner work clothing or personal articles to their homes. Only issued protective clothing should be allowed in the workplace. All laundry personnel should be thoroughly

TABLE III-5
CONTAMINATION OF THE SKIN WITH TNT
(in mg/dm²)

Washing Site	No. of Subjects	Conditions		
		After work	After shower	Before work
Uncovered sections of skin	22	1.68±0.4	0.087±0.03 p<0.01	0.047±0.016 p>0.5
Covered sections of skin	17	0.31±0.1	0.044±0.01 P<0.01	0.051±0.01 P>0.05

Adapted from reference 152

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TABLE III-6
AMOUNTS OF TNT ON DIFFERENT SURFACES

Surface	Amount of TNT (mg)			
	Swab 1	Swab 2	Swab 3	Swab 4
Palm of worker				
No. 1	0.012	0.008	ND*	ND
No. 2	0.024	0.008	ND	ND
No. 3	1.2	0.014	0.02	ND
Tubing	0.072	0.002	ND	ND
Wall	0.012	0.007	ND	ND
Window Glass	0.128	0.071	ND	ND

Adapted from reference 378

*ND = None Detected

informed of the nature of the contaminants and their potential adverse health effects.

The effectiveness of laundry soap in removing TNT from clothes was researched by Marchenko and coworkers [152]. They found up to 0.3 g of TNT/m³ of fabric in laundered work clothes. To determine the TNT on protective clothing, Osina and Dymova [378] soaked swatches in acetone for an hour to remove the compound from the fabric. They determined the amounts of TNT by spectrophotometry, after reacting the solution with potassium hydroxide, but did not report the results. Norwood [79] reported that total removal of TNT from highly contaminated clothes required prewashing with 3-5% sodium sulphite.

As another protective measure to minimize skin exposure by contaminated clothing, the employer should provide locker facilities for storing street clothing and personal belongings. A shower facility should be located so that workers wearing contaminated clothing do not have to enter the locker area. At the end of a shift, all work clothes, including undergarments, should be placed in a covered receptacle. All workers should shower before entering the locker area. One company [J Baliker, written communication, May 1979] planned to use a two-part change area for decontamination, separated by showers. All employees working with TNT would use the change facility. First, each person would deposit food, tobacco products, and chewing gum in a storage space outside the clean section of the change facility. Then, the employee would enter the clean side, remove all clothing, and receive a complete set of clean work clothes. Next, the worker would enter the transition area to put on boots, hardhat, gloves, and safety glasses or goggles. Finally, the employee would enter the work area through the

"dirty side" of the change facility. After the workshift, each person leaving an area where TNT is handled would enter the "dirty side" of the facility and reverse the procedure. The transition area would first be used to wash and store hardhat, gloves, safety glasses or goggles, boots, and tools. Then, all outer and inner work clothing would be removed and placed in hampers. After showering, each worker would put on street clothing and leave the area, picking up whatever items were left outside the change facility upon arrival.

To prevent accidental ingestion of TNT, workers should be told to wash their hands thoroughly with the recommended cleanser (liquid soap with 5-10% potassium sulfite and 5-10% wetting agent) before eating, touching tobacco products, or using toilet facilities. They should also be instructed to avoid touching the face and mouth with the hands, gloves, or other contaminated objects. Storing, dispensing, carrying, preparing, or using candy, food, beverages, tobacco, or chewing gum in TNT work areas should be prohibited. Areas where food is stored, prepared, and consumed must be kept free of contamination by TNT. Contaminated gloves, hardhats, and tools must be kept away from these areas. Handwashing and boot-cleaning facilities must be provided to prevent TNT contamination of the eating area.

When respirators are required, the employer should ensure that only NIOSH- or MSHA-approved respirators are used. A respiratory protective program to ensure that respirators are routinely inspected and properly cleaned, maintained, and stored, should be followed in accordance with 29 CFR 1910.134.

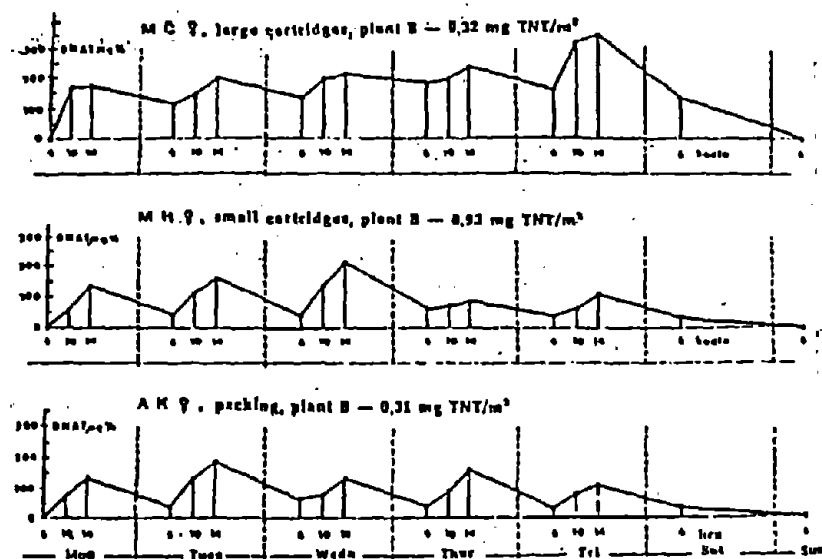
Biologic Monitoring of Exposure

Biologic monitoring of exposure to aromatic nitro compounds by either direct or indirect methods is important in the assessment of worker exposure to TNT. Direct methods measure the content of nitro compounds in the

urine [135]; other medical monitoring methods measure the concentration of methemoglobin in the blood [264]; the oxygen-carrying capacity of the blood [562]; or the activities of enzymes released from injured cells of the liver into the blood [264].

In a study of employee exposure in munitions and industrial explosives plants, Hassman and Hassmanova [135] measured the concentration of 2,6-dinitro-4-aminotoluene (DNAT) in the urine of 88 workers as an indication of exposure to TNT. The purpose of the study was to determine if the intensity of Webster's reaction [502], a colorimetric procedure to determine the presence of TNT in urine, can be correlated with the urinary concentration of DNAT, a metabolite of 2,4,6-TNT. The authors closely monitored DNAT concentration in the urine of nine persons by taking samples before the shift, in the middle of the shift, and after the shift for 5 consecutive workdays, and in the morning on the 2 following offdays. They concluded that DNAT elimination increases rapidly during exposure to TNT, decreases after cessation of exposure, and parallels the intensity of Webster's reaction in the urine. Thus, any TNT-monitoring program that measures DNAT in urine would have to ensure uniformity in terms of the time of collection of urine samples (see Figure III-8). Because of limited air-sampling data from the plants in which the employees worked during the study, the authors could not correlate statistically the DNAT concentration in urine with TNT concentration in the employees' breathing zones; the authors speculated that such a correlation could have been made if there had been a larger data base.

TNT-exposed workers at the Newport Army Ammunition Plant (NAAP) were studied by Morton [264] from August 1973 to April 1974. Several indirect biologic monitoring methods were used to identify employees that exhibited



Adapted from reference 135

FIGURE III-1. ELIMINATION OF DNAT IN THE URINE OF EMPLOYEES MC, MH, AND AK IN PLANT B

effects of TNT exposure. The procedures included estimation of the concentration of hemoglobin (Hb) in whole blood and the activities of serum glutamic oxaloacetic transaminase (SGOT) and lactic acid dehydrogenase (LDH) in blood serum. The study was conducted on 43 individuals exposed to TNT, and air samples for TNT were taken to correlate exposure with the results of the biologic tests. Air samples were taken with a Model G MSA pump and analyzed colorimetrically. The environmental data identified several areas with air concentrations of TNT in excess of 1.5 mg/m³, but no mention was made of what constituted an employee exposure to TNT or whether skin exposures were considered. The distribution of abnormal biologic tests correlated closely with production rates and TNT dust concentrations. Abnormal results were defined as being outside the range of "laboratory normals" in at least one of the tests performed. They were found in employees exposed to TWA concentrations of TNT dust of <1.5 mg/m³, the present OSHA limit. During the study, 27 biological abnormalities were found among 43 workers (see the section on Epidemiologic Studies in Chapter II). Employees with elevated activities of LDH in the sera returned to normal within 1-3 weeks after removal from exposure. The NAAP considers that normal values for Hb are >13.5g, SGOT<45 units, and LDH<102 units.

Sprout et al [562] recommended using the reflection oximeter to measure oxygen saturation levels of the blood as a method of determining exposure to aromatic nitro or amine compounds. Direct measurement of hemoglobin saturation with oxygen is more desirable than measuring the concentration of methemoglobin, because the hydroxylamine metabolites of nitrotoluenes may form iron-containing pigments other than methemoglobin. These pigments are inactive in oxygen transport, which further reduces the oxygen-carrying of the blood [562].

In addition to being accurate, the reflection oximeter test is simple to perform. A volume of 0.2 ml of arterial blood taken by capillary puncture of an earlobe is sufficient to make a determination. Standardizing and reading a sample takes <1 minute, and the authors reported an accuracy of $\pm 1\%$ at a true value of 90% oxygen saturation [562]. However, a precautionary note was expressed by Sprout et al [562] regarding the use of this method to determine the extent of hypoxemia; hypoxemia due to the oxidation of Hb takes time to develop. Consequently, if exposure is suspected, it is important to track the concentration of oxyhemoglobin in a person's blood at intervals of 20-30 minutes to determine if hypoxemia is developing. Otherwise, the employee might be dismissed from observation before hypoxemia and cyanosis developed fully. Furthermore, this test should be used only in conjunction with other tests to guarantee a safe environment for employees working with compounds that induce cyanosis.