6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring 2,4-DNT and 2,6-DNT, its metabolites, and other biomarkers of exposure and effect to 2,4-DNT and 2,6-DNT. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

The need to determine DNT in biological materials could arise from occupational exposure in the manufacture and processing of 2,4,6-TNT and from exposure to waste water and waste disposal sites associated with TNT manufacture. It has been noted (Jenkins et al. 1986) that “one of the Army’s most serious water pollution problems is the disposal of wash waters used to clean equipment and interior surfaces at munitions manufacturing and demilitarization facilities.” The same reference mentions the generation of large quantities of waste water from these facilities.

Although there are numerous occupational monitoring studies, a limited number of methods regarding the determination of DNT in biological samples is available in the literature. DNT has been determined in ocean floor fauna using thin layer chromatography (TLC) (Hoffsommer et al. 1972). Procedures have been described for the examination of swabs for traces of explosives, including 2,6-DNT using high-performance liquid chromatography (HPLC) with electrochemical detection at a pendent drop electrode (Lloyd 1983a). These techniques can be applied to biological materials such as skin surfaces exposed to explosives. DNT and its metabolites were determined in blood and urine by gas chromatography (GC) techniques (Turner et al. 1985; Woollen et al. 1985) and in urine by TLC (Woollen et al. 1985). Qualitative determination of DNT and its metabolites can also be performed after reduction to primary arylamines and subsequent coupling of diazo compounds to produce a colored complex (Smith et al. 1995).

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Dichloromethane is the solvent of choice for extracting DNT from water samples (EPA 1982a) and from wastes (EPA 1986a). Reversed-phase high-performance liquid chromatography (RP-HPLC) is attractive for the determination of DNT in waste water because it enables direct analysis of aqueous samples (Jenkins et al. 1986). A medium similar to the mobile phase used in this HPLC separation, i.e., 50/38/12 (v/v/v) water/methanol/acetonitrile, should be suitable for extracting DNT from low-lipid biological samples and for subsequent HPLC determination after sample cleanup.

Methods for the determination of the DNT in biological samples are given in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

The basic method for collecting DNT from the ambient atmosphere is adsorption on a solid phase, such as granular adsorbents (silica gel), filters, and impingers, followed by removal with solvents such as chloroform. Bubbler collectors can also be used for direct collection of analyte in a non-volatile solvent such as ethylene glycol. New instrumentation for the detection of 2,4-DNT and other explosives has recently been developed that will accept both air and surface particulate samples (Nacson et al. 1994). The instruments consist of capillary GC columns terminating in an electron-capture detector; the detection limit for 2,4-DNT is 20 ppt (Nacson et al. 1994). Also available is a portable version that is useful for a wide variety of applications, such as security checks, mail or passports, or in high-risk facilities (Nacson et al. 1994).

DNT is most commonly extracted with dichloromethane from water samples (EPA 1982a) and from wastes (EPA 1986a). A continuous countercurrent liquid-liquid extraction method is useful in extracting DNT from surface water samples (Deroux et al. 1996). The advantage of this method is that it is capable of extractions from large sample volumes and unfiltered natural water samples (Deroux et al. 1996). Supercritical fluid extraction (SFE) has been used with GC to reduce the preparation time of DNT in solids, such as soil (Francis et al. 1995). A sonic extraction-liquid chromatographic method has also been used for detection of 2,4-DNT in soils (Bauer et al. 1990; Griest et al. 1993). A simple screening method has been developed for the detection of 2,4-DNT in field soil samples that utilizes the spectrophotometer for identification by colorometrics after an initial reaction of the extract with potassium hydroxide and sodium sulfite (Jenkins and Walsh 1992).

The analysis of DNT is normally done by GC with a variety of detectors, including flame ionization detector (FIJI), electron capture detector (ECD), Hall electrolytic conductivity detector (HECD), thermionic specific

TABLE 6-1. Analytical Methods for Determining Dinitrotoluene in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine containing dinitrotoluene and metabolites	Hydrolysis of metabolites, extraction, derivatization	GC/MS	0.1 mg/L	NR	Turner et al. 1985
Urine containing dinitrotoluene and metabolites	Zinc-catalyzed reduction of DNT with hydrochloric acid to primary arylamines; diazotize and couple with *N*-(1-naphthyl)ethylene diamine to produce complex	SP	100 ng/mL	NR	Smith et al. 1995
Urine containing metabolites	Extraction with ethyl acetate	GC	0.1 mg/L	NR	Woollen et al. 1985
Urine	Extraction with ethyl acetate	TLC	0.1 mg/L[a]	NR	Woollen et al. 1985
Tissue/fluids	Extract with acetonitrile and filer with nylon membrane.	HPLC UV/VIS	0.4–0.5 ppm	–	Caton and Griest 1996
Plant tissue	Extract with sonication. Clean up on flonsil; alumina (liq. chrom.). Inject.	Reverse-phase LC UV/VIS	0.02–10 mg/mL	–	Larson 1998
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL[a]	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a
Blood	Extraction with toluene	GC	0.00001 mg/mL[a]	NR	Woollen et al. 1985
Ocean floor fauna	NR	TLC	NR		Hoffsommer et al. 1972

TABLE 6-1. Analytical Methods for Determining Dinitrotoluene in Biological Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL[a]	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a
Skin	Swab surface and insert into outlet port	GC/ECD	20 ppt (2,4-DNT)		Nacson et al. 1994

[a]Lowest detected concentration

ECD = electron capture detection; ED = electrochemical detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; MS = mass spectrometry; NR = not reported; SP = Spectrophotometry; TLC = thin layer chromatography

6. ANALYTICAL METHODS

detector (TSD), fourier transform infrared (FT-IR), or mass spectrometry (MS). It has been noted (EPA 1986b) that 2,4-DNT is "subject to erratic (gas) chromatographic behavior." When mass spectrometry is used to analyze water samples for DNT, electron impact (EI) is preferentially used because many structurespecific fragments will be formed, which can be used for identification of isomers (Feltes et al. 1990). To improve the accuracy of mass spectroscopic techniques in the identification of pollutants in aqueous and solid matrices, EPA has developed the method of isotope dilution (EPA 1989). Isotope dilution employs stable, isotopically labeled analogs of both 2,4- and 2,6-DNT to be used as internal standards in GUMS analysis (EPA 1989). Negative-ion chemical ionization has been shown to have a higher sensitivity and selectivity than EI, however, and should be used when determining traces of nitroaromatic compounds in complex aqueous mixtures (Feltes et al. 1990).

A sensitive and selective technique that has been used to identify trace amounts of 2,4-DNT and other explosive vapors is negative ion mobility spectrometry (Clark et al. 1995). This technique makes use of a tunable laser ionization source to produce characteristic negative ions, which can be used to identify the chemicals present (Clark et al. 1995). TLC and high-performance thin layer chromatography (HPTLC) have also been used to identify and quantify 2,4- and 2,6-DNT in soil and water samples from contaminated waste sites (Griest et al. 1993; Sohr et al. 1995; Steuckart et al. 1994). GC analysis is difficult because of the large amounts of humic acids present which cause overlap of matrix signals without cleanup; therefore, HPTLC can be a more advantageous method (Steuckart et al. 1994). Cleanup is not necessary with HPTLC, except in the analysis of soil samples (Steuckart et al. 1994).

A sensitive method for the analysis of DNT in drinking water has been developed using wide-bore fused silica capillary column GC with an ECD (Hable et al. 1991). The detection limits of this method are 0.04 μg/L for 2,4-DNT and 0.003 μg/L for 2,6-DNT; these detection limits are sensitive enough to meet the suggested requirements for EPA health advisories and water quality criteria.

For the determination of 2,4-DNT in munitions manufacture waste water, RP-HPLC was chosen by Jenkins et al. (1986) because it enables direct analysis of samples in aqueous solution without prior extraction, attains adequate detection limits without preconcentration, and avoids problems with analyte thermal instability. The detection limit for 2,4-DNT was 10 μg/L with a standard deviation of 3.4 μg/L for concentrations up to 250 μg/L. HPLC with photodiode array detection was used by Bouvier et al. (1995) to separate and quantitate 2,4- and 2,6-DNT in water samples. Recoveries ranged from 95 to 100%, and minimum detection limits were 0.041- 0.160 μg/L (Bouvier et al. 1995). A convenient method for analysis of 2,4- and 2,6-DNT in

contaminated soils used high performance liquid chromatography with minimal sample preparation (Preslan et al. 1993).

Methods for the determination of DNT in environmental samples are summarized in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 2,4-DNT and 2,6-DNT is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 2,4-DNT and 2,6-DNT.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The available methods for the determination of DNT and its metabolites in biological samples are inadequate. Although one method exists for determination of DNT and its metabolites in urine (Smith et al. 1995; Turner et al. 1985; Woolen et al. 1985), blood (Woolen et al. 1985), and skin (Lloyd 1983a; Nacson et al. 1994), there is a need for modem validated standard methods of analysis for such data in plant and animal tissues and exudates. Methods do exist for water and waste water (EPA 1982a, 1982b) and for solid wastes (EPA 1986a, 1986b, 1986c). The need also exists for good methods to determine DNT biomarkers in biological materials. The determination of this compound in plant and animal tissues and exudates would be useful to help determine exposure.

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Air	Ethylene glycol bubbler, filter, impinger	HPLC/UV	0.9 mg/m^3		NIOSH 1977
Air	Silica gel, desorb with chloroform	GC	0.1 mg/m^3	70–84	Hunt et al. 1980
Air	Collect sample and insert into analyzer	GC/ECD	20 ppt (2,4-DNT)		Nacson et al. 1994
Water	Extraction with dichloromethane	GC/ECD or TEA	ECD: 3.8×10^{-14} g/s (2,4-DNT); TEA: 1.77×10^{-11} g/s (2,4-DNT)		Feltes et al. 1990
Water	Extraction with dichloromethane	GC/MS (electron impact-full scan)	47 pg (2,6-DNT)		Feltes et al. 1990
Water	Extraction with dichloromethane; add methanol	GC/MS	5 ppb		Yinon 1996
Water	Adjust pH of spiking solution to >11 with NaOH; extraction with dichloromethane; add anhydrous sodium sulfate; filter; rotary evaporate	Liquid-liquid extraction with GC/MS	0.8 µg/L (2,4-DNT); 1.4 µg/L (2,6-DNT)	96 (2,4-DNT); 100 (2,6-DNT)	Yook et al. 1994
Water	Extraction with acetonitrile	HPLC/PDA	0.04–0.07 µg/L (2,4-DNT); 0.08–0.16 µg/L (2,6-DNT)	97–100 (2,4-DNT); 95–99 (2,6-DNT)	Bouvier et al. 1995

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Water	Counter current liquid-liquid extraction method	GC/MS (EI and PICI)	2 ng/L	89	Deroux et al. 1996
Water	Adjust to pH 12 and pump into column. Remove organic phase. Add to re-tirnate and adjust to pH 2 before pumping into column.	GC/MS	2 ng/L	89	Deroux et al. 1996
Water	Spike H2O sample with standards. Conduct solid-phase extraction (SPE). Elute and dry under nitrogen.	TLC (254 nm)	20 ng (scanner) 40 ng (visually)	diol-119-115.3 RP-18-100.6-102	Kessel and Hauch 1996
Aqueous	Mix sample with sodium chloride until salt dissolves. Concentrate extracts. Combine with 5.0 g/L $CaCl_2$ solution and inject.	HPLC (254 nm)	0.13 µg/L	–	Weisberg and Ellickson 1998 (modification to EPA Method 8330)
Drinking water	Extraction with 0.5 mL toluene; rotate 30 minutes at 15 RPM	GC/ECD	0.003 µg/L (2,6-DNT); 0.04 µg/L (2,4-DNT)	93–103 (2,6-DNT); 93–96 (2,4-DNT)	Hable et al. 1991
Drinking water	Elute initially with ethyl acetate, then with dichloromethane	GC/MS			Munch et al. 1993

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Groundwater and surface water	Extraction with dichloromethane; acidify water sample with HCl and extract with isobutylmethyl ketone; concentrate in rotary evaporator; dissolve residues in dichloromethane	TLC	10–20 ng/spot		Sohr et al. 1995
Groundwater	Extraction with dichloromethane	HPTLC-AMD	20 ng (2,4- and 2,6-DNT)		Steuckart et al. 1994
Groundwater	Expose three samples to different amounts of sunlight. Inject sample, loop.	HPLC/UV (254 nm)	0.01–0.1 µg/L	–	Spiegel and Welsch 1997
Groundwater	Extract with dichloromethane and dry over sodium sulfate.	HPLC	1–10 µgm/L	–	Preiß et al. 1996
	Extract with dichloromethane and dry over sodium sulfate. Add 1 mL acetonitrile.	NMR			
Waste water (for 2,4-DNT)	Diluted directly with methanol and acetonitrile	HPLC/UV	4.6 µg/L	NR	Jenkins et al. 1986
Waste water	Extraction with dichloromethane	GC/IDMS	10 µg/L	10 (2,4-DNT); 17 (2,6-DNT)	EPA 1980a

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Waste water	Extraction with dichloromethane, exchange to hexane	GC/ECD	NR		EPA 1982a
Waste water	Extraction with dichloromethane; EPA Method 8090	GC/MS	5.7 µg/L (2,4-DNT); 1.9 µg/L (2,6-DNT)		EPA 1986a
Waste water	Extraction with hexane	GC/ECD	25.0×10^{-5} µg (2,4-DNT)[b]; 62.5×10^{-6} µg (2,6-DNT)[b]	80 (2,4-DNT); 26 (2,6-DNT)	Hartley et al. 1981
Non-water miscible waste	Extraction with dichloromethane; EPA Method 8090	GC/ECD	2,000 µg/L (2,4-DNT); 1,000 µg/L (2,6-DNT)		EPA 1986a
Vapor	Solid samples placed in gas flow system	Negative ion mobility spectrometry (laser ionization source)	NR	NR	Clark et al. 1995
Biosludge	Extraction with sulfuric acid and dichloromethane	GC/TEA	0.05 mg/L	84	Phillips et al. 1983
Soil	Supercritical fluid extraction (SFE) with neat CO_2 and CO_2 with organic modifiers	GC/TDM	2.6 ppb		Francis et al. 1995

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Soil	Extraction with acetonitrile in ultrasonic bath; flocculate supernatant with $CaCl_2$; filter	SE/LC		95–97	Bauer et al. 1990
Soil	Dilution directly with methanol	HPLC/PDA	40–80 pg/µL		Emmrich et al. 1993
Soil (2,4-DNT)	Extraction with acetone; filter supernatant; react with potassium hydroxide and sodium sulfite	Spectrophotometry	2 µg/g		Jenkins and Walsh 1992
Soil	Grind soil; extraction with acetone in ultrasonic bath; centrifuge; add 5 mL toluene and remove acetone; dry toluene extract over anhydrous sodium sulfate	HPTLC/AMD	20 ng (2,4- and 2,6-DNT)		Steuckart et al. 1994
Soil	Extract with toluene, acetonitrile, methanol and collect in solid-liquid trap.	SFE/HPLC GC	–	100.6–101.5	Deuster et al. 1996
Soil, sediment, solid waste	Extraction	GC/MS	660 µg/kg		EPA 1986b
Soil, sediment, solid waste	Extraction with dichloromethane	GC/FT-IR	10 µg/L		EPA 1986c

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Soil, sediment, solid waste	Extraction	GC/FT-IR	10 µg/L	NR	Gurka et al. 1987
Soil/sediment	Air dry sample and homogenize and pass through sieve. Extract subsample with acetonitrile ultrasonically. Combine with 5.0 g/L $CaCl_2$ solution and inject.	HPLC (214 nm)	0.05 mg/kg	–	Weisberg and Ellickson 1998 (modification to EPA Method 8330)
Soil, water, and municipal sludges	Extraction with dichloro-methane; addition of isotopically labeled analog	GC/MS	10 µg/mL	NR	EPA 1989
Soil/compost	Acid leaching followed by sonic extraction	HPLC	0.055–0.248 mg/L[c]	NR	Griest et al. 1993
Soil/compost	Extraction with acetonitrile. Combine with $CaCl_2$. Derivatize with TFAA, then deactivate with H_2O.	HPLC	–	NR	Preslan et al. 1993
Soil/compost, leachates	Extract ultrasonically with acetonitrile. Filter with nylon membrane syringe filter.	HPLC uv/vis	0.4–0.5 ppm	–	Caton and Griest 1996
Materials exposed to DNT (bomb debris)	Swab with ethanol	HPLC/ED	5.6 µg/L	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983b

TABLE 6-2. Analytical Methods for Determining Dinitrotoluene in Environmental Samples (*continued*)

Sample matrix	Preparation method[a]	Analytical method[a]	Sample detection limit[a]	Percent recovery	Reference
Materials exposed to DNT	Swab surface and insert into outlet port	GC/ECD	20 ppt (2,4-DNT)		Nacson et al. 1994
Phenolic and nitroaromatic compounds	Inject sample, loop-separate using supercritical CO_2 as mobile phase.	SFC (230 nm)	oxidative-250 pg reductive-100 pg	–	Wallenborg et al. 1997
Explosives	Extract in acetonitrile and dilute in pH 7 buffer. Inject hydrostatistically and detect by uv.	MECC (214 nm)	0.55–0.74 mg/L	–	Oehrle 1996

2,6-dinitrotoluene (2,6-DNT) unless otherwise noted

[a]Analyses for both 2,4-dinitrotoluene (2,4-DNT) and
[b]Minimum detection to ECD
[c]Varied over course of experiment

AMD = automated multiple development; $CaCl_2$ = calcium chloride; CO_2 = carbon dioxide; ECD = electron-capture detection; ED = electrochemical detection; EI = electron ionization; FT-IR = fourier transform infrared; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HPTLC = high-performance thin layer chromatography; IDMS = isotope dilution mass spectrometry; LC = liquid chromatography; MECC = micellar electrokinetic capillary chromatography; MS = mass spectrometry; NaOH = sodium hydroxide; NR = not reported; PDA = photodiode array detection; PICI = positive ion chemical ionization; RPM = revolutions per minute; SE = solid extraction; SFC = super critical fluid chromatogaphy; TDM = thermal desorption modulator interface; TEA = thermal energy analysis; TLC = thin layer chromatography; UV = ultraviolet absorption

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Methods for Determining Parent Compounds and Degradation Products in Environmental Media. DNT can be analyzed in water, air, and waste samples with reasonable selectivity and sensitivity (EPA 1986a, 1986b, 1986c, 1989; Gurka et al. 1987; Nacson et al. 1994; NIOSH 1977; Yinon 1996; Yook et al. 1994). Therefore, there is a reasonable database in this area.

There exists an ongoing effort to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988). The overall goal is to detect and measure quantitatively organic compounds at 0.1 μg/L in drinking water, 1 μg/L in surface waters, and 10 μg/L in effluent waters. Analytes well include numerous semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (boiling point <150°C). A comprehensive review of the literature leading up to these efforts has been published (Pellizzari et al. 1985). It may be anticipated that improved methods for the determination of semivolatile DNT isomers in environmental samples may be developed as part of this effort.

6.3.2 Ongoing Studies

No ongoing studies were identified on analytical methods for DNT and its metabolites.