7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring DNTs, their metabolites, and other biomarkers of exposure and effect to DNTs. 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.

7.1 BIOLOGICAL MATERIALS

The need to determine DNTs 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 DNTs in biological samples are available in the literature. DNTs have been determined in ocean floor fauna using thin layer chromatography (TLC) (U.S. Navy 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. DNTs and their 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 DNTs and their metabolites can also be performed after reduction to primary arylamines and subsequent coupling of diazo compounds to produce a colored complex, which absorbs light at 550 nm (Smith et al. 1995).

Dichloromethane is the solvent of choice for extracting DNTs from water samples (EPA 1982a) and from wastes (EPA 1986a). Reversed-phase high-performance liquid chromatography (RP-HPLC) is attractive for the determination of DNTs 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 DNTs from low-lipid biological samples and for subsequent HPLC determination after sample cleanup.

In a study conducted by Honeychurch et al. (2003), screen-printed carbon electrodes (SPCEs) were used as disposable sensors for the measurement of 2,6-DNT using a stripping voltammetric method. Voltammograms showed the appearance of two reduction peaks that corresponded to the formation of the hydroxylamine derivatives from the reduction of the nitro groups and one oxidation peak possibly from the formation of the nitrosamines. The detection limit was 161 ng/mL (0.161 mg/L) and the sensors were evaluated by determining concentrations of 2,6-DNT in spiked and unspiked potable water, saliva, and dust wipes. Percent recoveries for spiked samples were 92.8–113% for potable water, 41.0–52.0% for saliva, and 67.6–79.1% for dust wipes. 2,6-DNT was not detected in unspiked samples. This new study indicates that SPCEs could possibly be used in the analytical detection of DNTs in occupational settings.

Methods for the determination of the DNTs in biological samples are given in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

The basic method for collecting DNTs 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. Other instrumentation for the detection of 2,4-DNT and other explosives 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 (ECD); the detection limit for 2,4-DNT is 20 ppt (2×10^{-5} ppm) (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).

Table 7-1. Analytical Methods for Determining Dinitrotoluenes in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine containing DNTs and metabolites	Hydrolysis of metabolites, extraction, derivatization	GC/MS	0.1 mg/L	NR	Turner et al. 1985
Urine containing DNTs and metabolites	Zinc-catalyzed reduction of DNT with hydrochloric acid to primary arylamines; diazotize and couple with *N*-(1-naphthyl)ethylene diamine to produce a colored complex with characteristic adsorption at 550 nm	UV/VIS	100 ng/mL (0.1 mg/L)	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
Plant tissue	Extract with sonication; cleanup on flonsil; alumina (LC); inject	Reverse-phase LC UV/VIS	0.086–0.109 mg/L	98.2	U.S. Army 1998
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL[a] (0.0056 mg/L)	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a
Blood	Extraction with toluene	GC	0.00001 mg/mL[a] (0.01 mg/L)		Woollen et al. 1985
Ocean floor fauna	NR	TLC	NR		U.S. Navy 1972
Skin	Swab with ethanol	HPLC/ED	5.6 ng/mL[a] (0.0056 mg/L)	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983a

[a]Lowest detected concentration.

DNT = dinitrotoluene; ECD = electron capture detection; ED = electrochemical detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; NR = not reported; TLC = thin layer chromatography; UV/VIS = ultra-violet/visible spectroscopy

Sylvia et al. (2000) developed a Surface-Enhanced Raman Spectroscopy (SERS) to potentially detect 2,4-DNT vapors above TNT-based landmines. This method of detection exhibited reproducible results, sensitive detection levels of ≤5 ppb (0.005 ppm) 2,4-DNT, and spectra that demonstrated high signal-to-noise ratios collected in 30 seconds. While only preliminary blind testing was done, vapor-phase SERS is a promising instrumental method for the detection of explosive vapors.

Albert and Walt (2000) developed a novel cross-reactive optical microsensor for high-speed detection of low-level explosives and explosive-like vapors. This porous silica-based sensor, was used to detect DNTs in water vapor at a level of 23 ppb in clean dry air.

Likewise, a field developed instrument with artificial cross-reactive optical sensors was employed for the detection of 2,4-DNT vapors in spiked soil and aqueous and ground samples. Two types of fluorescence based vapor sensors were developed: one sensor was semiselective for the nitroaromatic compounds (NAC), while the other was non-specific and cross reactive. The fluorescent sensors were exposed to multiple solvents and were validated/calibrated by making measurements of known and estimated concentrations of 2,4-DNT. The system has demonstrated the ability to detect 120 ppb 2,4-DNT vapor in blind humidified samples and has been exclusively used in field tests (Albert et al. 2001).

DNTs are 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 DNTs 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). A sonic extraction-liquid chromatographic method has 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).

A biosensor has been developed by Smirnova et al. (2004) for the detection of DNTs in soil and groundwater. The genetic system from the *Burkholderia sp.* strain DNT was examined and used to develop the prototype cell-based biosensor. The central element of the biosensor was found to be two crystal structures of the transcriptional regulator DntR with acetate and thiocyanate occupying the inductor-binding cavity. Analysis of this biosensor was done to model how 2,4-DNT might bind to DntR and to ultimately make conclusions on how the specificity of the biosensor might be enhanced.

The analysis of DNTs is normally done by GC with a variety of detectors, including flame ionization detector (FID), electron capture detection (ECD), Hall electrolytic conductivity detector (HECD), thermionic specific detector (TSD), fourier transform infrared (FT-IR), thermal energy analyzer (TEA), 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 structure-specific 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 GC/MS 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).

The quenching of the photoluminescence of porous silicone films in a flowing air stream once the films were exposed to DNTs was monitored and the quantity of DNTs was detected in a study by Content et al. (2000). This detection was achieved by catalytic oxidation of DNTs. The detection limit of 2 ppb was observed for 2,4-DNT.

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 DNTs 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 (4×10^{-5} mg/L) for 2,4-DNT and 0.003 μg/L (3×10^{-6} mg/L) for 2,6-DNT; these detection limits are sensitive enough to meet the suggested requirements for EPA health advisories and water quality criteria.

Also, HPLC with UV-detection for nitroaromatic compounds and amperometric detection for aminoaromatic and phenolic compounds were used by Spiegel and Welsch (1997) for the monitoring of

the degradation products of explosives in groundwater. These methods of detection were reported to be extremely sensitive. 2,4-DNT and 2,6-DNT were observed to be nearly completely metabolized as seen by their decrease in the chromatograms. Spiegel et al. (2005) found that HPLC-EC was more effective in detecting trace compounds, whereas HPLC-UV was found to be effective in detecting chemicals in highly contaminated groundwater. Detection limits ranged from 1 to 40 pg/L for HPLC-EC.

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 (0.01 mg/L) with a standard deviation of 3.4 μg/L (0.034 mg/L) for concentrations up to 250 μg/L (0.25 mg/L). 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). Likewise, DNTs were also measured in field soil samples by using a method that included the fortification of DNTs in the soil, recovery of DNTs by pressurized fluid extraction, and measurement of DNTs by gas and liquid chromatography-MS (Campbell et al. 2003). Compared with the former EPA method SW-846 8330, this method provided a quicker time frame needed to complete an extraction (15 minutes as opposed to 18 hours) and MS confirmation of the analytes. The limit of quantitation for 2,4-DNT was 0.05 μg/g.

Methods for the determination of DNTs in environmental samples are summarized in Table 7-2.

7.3 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 DNTs is available. Where adequate information is not available, ATSDR, in conjunction with 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 DNTs.

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

Table 7-2. Analytical Methods for Determining Dinitrotoluenes in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Silica gel, desorb with chloroform	GC	0.1 mg/m^3 (1x10^{-4} mg/L)	70–84	Hunt et al. 1980
Air	Dispersed on glass beads, glass tube, and Flowmeter/mixer; exposure to dry synthetic air	FT-IR/MCT detector, NMR	2 ppb (0.002 ppm) (2,4-DNT)		Content et al. 2000
Air	Collect sample and insert into analyzer	GC/ECD	20 ppt (2x10^{-5} ppm)		Nacson et al. 1994
Air	Collect sample and insert into analyzer	Fluorescent sensing film	(2,4-DNT) NR		Xin et al. 2011
Water	Extraction with dichloromethane	GC/ECD or TEA	ECD: 3.8x10^{-14} g/s (2,4-DNT); TEA: 1.77x10^{-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 (0.005 ppm)		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 (8x10^{-4} mg/L)(2, 4-DNT); 1.4 μg/L (1.4x10^{-4} mg/L) (2,6-DNT)	96 (2,4-DNT); 100 (2,6-DNT)	Yook et al. 1994
Water	Counter current liquid-liquid extraction method	GC/MS (EI and PICI)	2 ng/L (2x10^{-6} mg/L)	89	Deroux et al. 1996
Water	Adjust to pH 12 and pump into column; remove organic phase; add to retirnate and adjust to pH 2 before pumping into column	GC/MS	2 ng/L (2x10^{-6} mg/L)	89	Deroux et al. 1996
Water	Spike H_2O sample with standards; conduct 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 Hauck 1996

Table 7-2. Analytical Methods for Determining Dinitrotoluenes in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Extraction with 0.5 mL toluene; rotate 30 minutes at 15 RPM	GC/ECD	0.003 µg/L ($3x10^{-6}$ mg/L) (2,6-DNT); 0.04 µg/L ($4x10^{-5}$ mg/L) (2,4-DNT)	93–103 (2,6-DNT); 93–96 (2,4-DNT)	Hable et al. 1991
Groundwater and surface water	Extraction with dichloromethane; acidify water sample with HCl and extract with isobutyl-methyl 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)		Steukart et al. 1994
Groundwater	Expose three samples to different amounts of sunlight; inject sample, loop	HPLC/UV (245 nm)	0.01–0.1 µg/L ($1x10^{-5}$–$1x10^{-4}$ mg/L)	–	Spiegel and Welsch 1997
Groundwater	Salting-Out Extraction (SOE)	HPLC/EC	1–40 pg/L	–	Spiegel et al. 2005
Waste water (for 2,4-DNT)	Diluted directly with methanol and acetonitrile	HPLC/UV	4.6 µg/L (0.0046 mg/L)	NR	Jenkins et al. 1986
Waste water	Extraction with dichloromethane	GC/IDMS	10 µg/L (0.01 mg/L)	10 (2,4-DNT); 17 (2,6-DNT)	EPA 1980
Waste water	Extraction with dichloro-methane, exchange to hexane	GC/ECD	NR		EPA 1982a
Waste water	Extraction with dichloromethane; EPA Method 8090	GC/MS	5.7 µg/L (0.0057 mg/L) (2,4-DNT); 1.9 µg/L (0.0019 mg/L) (2,6-DNT)		EPA 1986a
Non-water miscible waste	Extraction with dichloromethane; EPA Method 8090	GC/ECD	2,000 µg/L (2 mg/L) (2,4-DNT); 1,000 µg/L (1 mg/L) (2,6-DNT)		EPA 1986a

Table 7-2. Analytical Methods for Determining Dinitrotoluenes in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Biosludge	Extraction with sulfuric acid and dichloromethane	GC/TEA	0.05 mg/L	84	Phillips et al. 1983
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 (0.04–0.08 mg/L)		Emmrich et al. 1993
Soil	Extraction with acetone; filter supernatant; react with potassium hydroxide and sodium sulfite	Spectro-photometry	2 μg/g (2,4-DNT)		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	Fortification, extraction	HPLC-DAD, LC/MS, GC/MS	0.05 μg/g		Campbell et al. 2003
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 (0.01 mg/L)		EPA 1986c
Soil, sediment, solid waste	Extraction	GC/FT-IR	10 μg/L (0.01 mg/L)	NR	Gurka et al. 1987
Soil, water, and municipal sludges	Extraction with dichloro-methane; addition of isotopically labeled analog	GC/MS	10 μg/mL (10 mg/L)	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

Table 7-2. Analytical Methods for Determining Dinitrotoluenes in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Materials exposed to DNT (bomb debris)	Swab with ethanol	HPLC/ED	5.6 µg/L (0.056 mg/L)	97 (2,6-DNT); 93 (2,4-DNT)	Lloyd 1983b
Materials exposed to DNT	Swab surface and insert into outlet port	GC/ECD	20 ppt ($2x10^{-5}$ mg/L) (2,4-DNT)		Nacson et al. 1994
Phenolic and nitroaromatic compounds	Inject sample, loop-separate using super-critical 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

[a]Analyses for both 2,4-DNT and 2,6-DNT unless otherwise noted.
[b]Minimum detection to ECD.
[c]Varied over course of experiment.

AMD = automated multiple development; $CaCl_2$ = calcium chloride; CO_2 = carbon dioxide; DAD = diode array detection; DNT = dinitrotoluene; 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; MCT = mercury cadmium telluride; MECC = micellar electrokinetic capillary chromatography; MS = mass spectrometry; NaOH = sodium hydroxide; NMR = nuclear magnetic resonance; NR = not reported; PDA = photodiode array detection; PICI = positive ion chemical ionization; RPM = revolutions per minute; SE = solid extraction; SFC = super critical fluid chromatography; SFE = supercritical fluid extraction; SPE = solid-phase extraction; TDM = thermal desorption modulator interface; TEA = thermal energy analysis; TFAA = trifluoroacetic anhydride; TLC = thin layer chromatography; UV = ultraviolet absorption

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The available methods for the determination of DNTs and their metabolites in biological samples are inadequate. Although one method exists for determination of DNTs and their 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 modern 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). A method that employs the use of SPCEs has the potential to be useful for examining biological samples such as potable water, saliva, and dust wipes (Honeychurch et al. 2003). 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.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. DNTs 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; 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, l μg/L in surface waters, and 10 μg/L in effluent waters. Analytes include numerous semivolatile compounds and some compounds that are only "semi-soluble" in water, as well as volatile compounds (boiling point <150 °C). 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.

7.3.2 Ongoing Studies

No ongoing studies regarding analytical methods sponsored by NIH or EPA were identified for DNTs.