6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 1,3-DNB and 1,3,5-TNB in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify 1,3-DNB and 1,3,5-TNB. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect I,3-DNB and 1,3,5-TNB in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter may be 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 may be included that refine previously. used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

The data on analytical methods for detecting 1,3-DNB and 1,3,5-TNB and their metabolites in biological media are very limited. The few methods that have been used are discussed in the following section and summarized in Table 6-l.

1,3-DNB and its metabolites have been determined in the blood and urine of rodents fed the compound (Bailey et al. 1988; McEuen and Miller 1991; Nystrom and Rickert 1987). The methods to detect 1,3-DNB include high-resolution gas chromatography (HRGC) with electron capture detection (ECD), high-performance liquid chromatography (HPLC) with radioactivity detection (RAD) (for radiolabeled compounds) or ultraviolet (UV) detection or liquid scintillation counting (LSC) (for radiolabeled compounds), gas chromatography (GC) with mass spectrometry (MS), and spectrophotometry. It should be noted that the HPLC/RAD method is not suitable for the determination of 1,3-DNB and its metabolites in humans since it requires exposure to radiolabeled compounds. A reported method for quantitating 1,3-DNB and its metabolites in blood and urine by HRGC/ECD has a limit of detection in the low ppb range, and both recovery (≈110%) and precision (±3% coefficient of variation [CV]) of the method were excellent (Bailey et al. 1988). The reported methods based on HPLC separation and detection/quantification of radioactivity (McEuen and Miller 1991; Nystrom and Rickert 1987) are not suitable for monitoring human exposure because they depend

TABLE 6-1. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (1,3-DNB and metabolites)	Extraction with ethyl acetate; centrifugation	HRGC/ECD	10 µg/L	110–111	Bailey et al. 1988
Blood (1,3-DNB and metabolites)	Extraction with methanol and centrifugation; elution from reverse-phase column with potassium phosphate/methanol	HPLC/UV/LSC	No data	No data	McEuen and Miller 1991
Blood (metabolites)	Extraction with methanol and centrifugation; evaporation; redissolution in water and extraction with ethyl acetate; H_2O removed (anhydrous Na_2SO_4) and concentration; separation by TLC	GC/MS	No data	No data	McEuen and Miller 1991
Urine (1,3-DNB and metabolites)	Elution from reverse-phase column with methanol/potassium phosphate/ tetrabutylammonium hydrogen sulfate	HPLC/UV/LSC	No data	No data	McEuen and Miller 1991
Urine (metabolites)	Extraction sample with ethyl acetate; separation by reverse-phase HPLC; derivatization with N-methyl-N-trimethyl-siyltrifluoroacetamide	GC/MS	No data	No data	McEuen and Miller 1991
Urine (1,3-DNB and metabolites)	Centrifugation; elution from reverse-phase columns with sodium phosphate/ acetonitrile	HPLC/RAD GC/MS	No data No data	No data No data	Nystrom and Rickert 1987

TABLE 6-1. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (NB)	Addition of concentrated HCl and Zn dust; addition of phenol red indicator; pH adjustment to 9.0 with NaOH solution; dilution and centrifugation; removal of supernatant and addition of phosphate buffer; pH adjustment to neutrality; reaction with sodium salt of 1,2-naphthoquinone-4-sulfonic acid; extraction with CCl_4; centrifugation; removal and filtration of organic layer	Spectrophotometry	0.8 mg/L	62–78	Dangwal and Jethani 1980
Skin or clothing	Skin wiped with isopropanol swabs or contaminant from clothing transferred by vacuuming onto membrane filters; extraction with acetonitrile and purification by HPLC.	HRGC/TEA	No data	No data	Lloyd 1991
Handswab, standards (1,3-DNB)	Hand wiped with dry swab; extraction with methanol/potassium phosphate	HPLC/EC (PMDE)	10 pg/inj (standards)	No data	Lloyd 1983
Handswab (1,3,5-TNB)	Hand wiped with swab; swab extraction with methyl *tert*-butyl ether; centrifugation; evaporation of supernatant; redissolution in methyl *tert*-butyl ether in pentane; clean-up on Amberlite XAD-7® column, elution with ethyl acetate; concentrate	HRGC/TEA HRGC/ECD	pg-low ng pg-low ng	No data No data	Douse 1985

CCl_4 = carbon tetrachloride; EC = electrochemical detection; ECD = electron capture detection; GC = gas chromatography; HCl = hydrogen chloride; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; inj = injection; LSC = liquid scintillation counting; MS = mass spectrometry; NaOH = sodium hydroxide; Na_2SO_4 = sodium sulfate; NB = nitrobenzene; PMDE = pendant mercury drop electrode; RAD = radiation absorbed dose; TEA = thermal energy analyzer; TLC = thin layer chromatography; UV = ultraviolet detector; Zn = zinc

on exposure to a radiolabeled dose of 1,3-DNB. GC/MS is a sensitive and highly selective method of detecting 1,3-DNB in blood and urine but has only been used for qualitative confirmation of the compound (McEuen and Miller 1991). It is possible that a modification of the method could be used for quantification of 1,3-DNB and its metabolites in these matrices. A spectrophotometric method for determining nitrobenzene in urine has been developed (Dangwal and Jethani 1980). This method could also be used to determine 1,3-DNB and 1,3,5-TNB in urine because it is somewhat selective for many nitro and amino benzene-based compounds. However, it is not as useful as HRGC/ECD because it is not as selective, and the sensitivity is several orders of magnitude less (ppm). An enzyme-linked immunosorbent assay (ELISA) has been proposed for the determination of 1,3-DNB in biological samples (Miller et al. 1991). The method showed good specificity and comparable recovery of 1,3-DNB from blood samples when compared with HPLC/UV and HPLC/radiochemical detection. No information was located that specifically discussed the detection of 1,3,5-TNB and metabolites in blood and urine by any method.

Methods have been developed for the detection of both 1,3-DNB and 1,3,5-TNB in extracts from hand-swab samples. The methods employ HRGC/ECD, HRGC/thermal energy analyzer (TEA), and HPLC/electrochemical detection (EC). Data are inadequate for a comparison of the sensitivity and reliability of these methods. Both HPLC and HRGC are effective in separating the analyte from other nitro compounds and contaminants (Douse 1985; Lloyd 1983). Detection by TEA (Douse 1985) and EC using a pendant drop mercury electrode (PDME) (Lloyd 1983) is more selective than ECD (Douse 1985). All three detectors were sensitive to ppb levels of analyte. The PDME has a unique advantage in that a new mercury drop can be formed between samples, and therefore, this detector is not subject to degeneration from contamination build-up. This makes the PDME highly reproducible (precision of 1.8% CV).

6.2 ENVIRONMENTAL SAMPLES

A large variety of methods has been described for the detection of 1,3-DNB and 1,3,5-TNB in environmental samples. These include GC or HRGC (combined with ECD, TEA, MS, nitrogen-phosphorus detection [NPD], or flame ionization detection [FID]), and HPLC (combined with UV and/or photoconductivity [PC] detection). Other methods that do not need chromatographic separation before quantitation, including MS, cyclic and differential pulse voltammetry, spectrophotometry, and assays based on chemical oxygen demand (COD) and total organic carbon (TOC), have also been used

or tested. Table 6-2 contains a summary of several representative methods for determining 1,3-DNB and 1,3,5-TNB in various environmental media. Methods in which nitrobenzene was the analyte have been included when the methods apply to 1,3-DNB and 1,3,5-TNB as well. For several of the methods included in Table 6-2, nitrobenzene is the analyte being investigated, although the method should also be useful for analysis of 1,3-DNB and/or 1,3,5-TNB (when nitrobenzene data were used, this is indicated in the Sample column).

The few methods located for analysis of nitro compounds in air have not been well characterized for 1,3-DNB or 1,3,5-TNB. Only one method considered the analysis of 1,3-DNB specifically. The remainder were for analysis of nitrobenzene but could also be applied to the analysis of 1,3-DNB or 1,3,5-TNB. Most air samples are preconcentrated by collection on a solid sorbent prior to measurement; however, grab samples or liquid impingers have been used in some methods for the collection of 1,3-DNB. HRGC and GC with FID have been used to measure 1,3-DNB and nitrobenzene in air samples (Andersson et al. 1983; Cooper et al. 1986; Kebbekus and Bozzelli 1982). Under the experimental conditions used, reliability was adequate, with accuracy ranging from 52 to 84% and precisions ranging from 6 to 30% CV (Andersson et al. 1983; Kebbekus and Bozzelli 1982). The lower recoveries and precisions were obtained with nitrobenzene (Kebbekus and Bozzelli 1982) and could be substantially different for 1,3-DNB and 1,3,5-TNB. However, tests with 1,3-DNB and 1,3,5-TNB would have to be conducted to determine reliability parameters for these compounds. Sensitivity for HRGC/FID using nitrobenzene as the analyte was in the low ppt (Kebbekus and Bozzelli 1982). A comparison of HRGC with either NPD or FID showed that NPD was far more selective for nitro compounds than FID (Cooper et al. 1986). The detection limit for HRGC/NPD under the conditions used was in the. low ppb, and the authors could not quantify nitrobenzene using HRGCYFID. A spectrophotometric method has been developed for detection of nitro and amino benzene-based compounds in air (Dangwal 1981). Since the method cannot differentiate between the various nitro- and amino-benzene compounds, it is substantially less selective than other available methods. The sample preparation is more complex than with other tested methods and involves extraction with carbon tetrachloride, a potentially hazardous chemical. In addition, the sensitivity is several orders of magnitude less (ppm) than the’sensitivity of the HRGC and GC methods.

Both GC (high and low resolution) and HPLC may be used to separate nitrobenzene compounds in water. The most common detector for HPLC analysis is UV. For the GC methods, several detectors have been used, including NPD, ECD, FID, TEA, and MS. The sensitivity of the methods varies from

TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (1,3-DNB)	Collection on personal sampler containing Amberlite XAD-2; desorption with diethyl ether	GC/FID	No data	79–84	Andersson et al. 1983
Air (NB)	Collection on Tenax; thermal desorption	HRGC/FID	0.01 ppb	52	Kebbekus and Bozzelli 1982
Air (NB)	Collection in ethanol; reaction with concentrated HCl and zinc dust; react with sodium salt of 1,2-naphtho-quinone-4 sulfonic acid at pH 8.0; extraction with CCl_4; concentration; separation by paper chromatography; extraction of fractions with CCl_4	Spectrophotometry	10µg	93	Dangwal 1981
Gas effluents (NB)	Collection in stainless steel container or on silica gel traps; dilution of canister sample with N_2; desorption of silica gel with ethanol	HRGC/FID HRGC/ECD	No data 7.1–57 ppb	No data No data	Cooper et al. 1986
Water (1,3-DNB, 1,3,5-TNB)	Addition of internal standard; extraction with CH_2Cl_2; or collection on Amberlite XAD-2, XAD-4, or XAD-8; column and dried; extraction with CH_2C_{l2}; extract dried over anhydrous Na_2SO_4; concentration and add redissolution in methanol	HRGC/ECD HRGC/TEA HRGC/MS	No data No data No data	No data No data No data	Feltes et al. 1990
Water (1,3,5-TNB)	Extraction with toluene containing internal standard	GC/NPD	10 µg/L	83–102	Army 1986

TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water (1,3,5-TNB)	Filtration; injection of sample directly or dilution and injection; elution from reverse-phase HPLC column with methanol/water	HPLC/UV	No data	94–102	Army 1986
Tap water (1,3-DNB and 1,3,5-TNB)	Collection on Amberlite XAD® resin; elution with ethyl acetate	HRGC/ECD	<0.1 µg/L	80–88 (1,3-DNB); 74–89 (1,3,5-TNB)	Richard and Junk 1986
Tap water, waste water effluents (NB)	Extraction with Freon-TF; concentration	GC/FID	0.12 µg/L	97–100	Austern et al. 1975
Waste water effluents (1,3-DNB)	Addition of internal standard; elution from Sep-Pak C_{18} reverse-phase column with methanol/water	HPLC/UV	~0.2 mg/L	No data	Army 1983
Surface water (1,3,5-TNB)	Collection on Amberlite XAD-2/4/8; dry; desorption with CH_2Cl_2 ; dry on anhydrous Na_2SO_4; solvent exchange to methanol; concentration; elution from reverse-phase column with methanol/water	HPLC/UV	50 ng/L	85–105	Feltes and Levsen 1989
Groundwater (1,3-DNB and 1,3,5-TNB)	Collection on Hayesep R solid sorbent cartridge; elution with acetone; concentration; addition of internal standard; dilution with methanol/water; elution from HPLC column with methanol/water	HPLC/UV/UV/PC	No data	No data	Army 1989
Groundwater, soil/sediment, solid waste (NB)	Sample extraction and clean-up methods recommended by EPA for the specific matrix	GC/MS	1.9 µg/L	35–114 (water); 23–120 (soil)	EPA 1986a

TABLE 6-2. Analytical Methods for Determining 1,3-DNB and 1,3,5-TNB in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil (1,3-TNB)	Sample dried, sieved and ground; extraction with ultrasonication in acetonitrile containing internal standard; dilution with aqueous $CaCl_2$; filtration; elution from reverse-phase column with methanol/water	HPLC/UV	0.11 µg/g (1,3-DNB); 0.29 µg/g (1,3,5-TNB)	105 (1,3-DNB); 119 (1,3,5-TNB)	Bauer et al. 1990; Jenkins et al. 1989
Wine, beer, cider (NB)	Direct measurement	DPV CV	0.5 mg/L	No data	Lorenzo et al. 1988

$CaCl_2$ = Calcium chloride; CH_2Cl_2 = Dichloromethane (methylene chloride); CCl_4 = Carbon tetrachloride; CV = Cyclic voltammetry; DPV = Differential pulse voltammetry; ECD = Electron capture detection; EPA = Environmental Protection Agency; FID = Flame ionization detection; GC = Gas chromatography;HCl = Hydrochloric acid; HPLC = High-performance liquid chromatography; HRGC = High-resolution gas chromatography; MS = Mass spectrometry; N_2 = Nitrogen gas; Na_2SO_4 = Sodium sulfate; NB = Nitrobenzene; NPD = Nitrogen-phosphorus detection; PC = Photoconductivity; TEA = Thermal energy analyzer; UV = Ultraviolet detector

sub to a mid-ppb range depending on the method, contamination level of the sample, efficiency of the extraction procedure, selectivity of the method, specific analyte (nitrobenzene, 1,3-DNB, or 1,3,5-TNB), and other method variables. The sensitivity of the GC-based methods is in the low-ppb range, with the limited data suggesting that ECD and FID may be slightly more sensitive than the other tested detectors (Austem et al. 1975; Richard and Junk 1986). However, TEA (Feltes et al. 1990), nitrogen-phosphorus (Army 1986), and MS (EPA 1986a; Feltes et al. 1990; Stemmler and Hites 1987) detectors are considered much more selective. GC/MS using a low-resolution instrument in electron ionization mode is the method recommended by EPA (EPA 1986a) because of its selectivity and sensitivity. However, other studies have shown that negative chemical ionization (NCI) and electron capture NCI are more sensitive and reliable than the EPA method (Feltes et al. 1990; Stemmler and Hites 1987). HPLC provides an alternative to GC-based methods (Army 1983, 1989; Feltes and Levsen 1989). The HPLC methods available are sensitive (detection limits ranging from low ppt to low ppb), relatively selective, reproducible, and reliable, in that they maintain the integrity of samples (sample decomposition may occur in heated zones of GC injectors). HPLC is also simple and rapid, requiring little sample preparation. A modification of the HPLC/UV method couples two UV detectors and a PC detector (Army 1989). This arrangement of detectors can improve the selectivity of HPLC substantially. An MS technique that allows direct injection of a water sample has also been tested (Yinon and Laschever 1982). The detection limit was only in the low-ppm range, but its selectivity makes it a good method for screening samples for further analysis. Assays based on COD and TOC (Roth and Murphy 1978) are well-established standard methods for determining organic pollution in water, but they are not selective for nitrobenzene compounds.

HPLC/UV was the only method located for measuring nitrobenzene, 1,3-DNB, and 1,3,5-TNB in soil (Army 1985a; Bauer et al. 1990; Jenkins and Grant 1987; Jenkins et al. 1989). This method, developed by the Army, has been extensively tested and has been proven to be selective and reliable, giving high recoveries and good precision for complex samples. Sensitivity in the low-ppm range has been reported. A similar method, with less rigid sample clean-up, had recoveries for nitrobenzene that varied widely (Grob and Cao 1990). This shows the importance of sample extraction and clean-up with regard to results when the matrix is complex.

Cyclic voltammetry and differential pulse voltammetry have been used to analyze wine, beer, and cider for nitrobenzene (Lorenzo et al. 1988). While no detection limits were reported, amounts as low as 0.5 mg/L were easily detected and precision was excellent (±5% CV). An advantage of this method is

that the analyte can be measured by direct insertion of the electrode in the solution. The method should also apply to the detection of 1,3-DNB and 1,3,5-TNB in solutions because it is based on polarographic determination of the nitro group. However, it is not as selective as HPLC- and GC-based methods. MS/MS has been investigated as a screening method for explosives (McLuckey et al. 1985), but no data on the sensitivity and reliability of this method were available. A supercritical fluid capillary chromatographic method with FID detection has been proposed for the determination of a broad range of compounds (including nitroaromatics) in solid wastes (Pospisil et al. 1991). The method was used to chromatograph over 270 compounds on a single column within 1 hour.

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 1,3-DNB and 1,3,5-TNB 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 1,3-DNB and 1,3,5-TNB.

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 or eliminate 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.

Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Very few methods were located that could be used to determine exposure to 1,3-DNB or 1,3,5-TNB in humans. A spectrophotometric method exists (Dangwal and Jethani 1980) but is selective for nitro and amino benzene-based compounds, not for 1,3-DNB and 1,3,5-TNB specifically. The best methods for determination of exposure to 1,3-DNB and 1,3,5-TNB are HRGUECD (Bailey et al. 1988) and GC/MS (McEuen and Miller 1991). To date, only HRGUECD has been used quantitatively, and only

for detection of 1,3-DNB and metabolites in blood and urine. The method has a detection limit of 10 ppb, a recovery of 110% and a coefficient of variation of 23% (Bailey et al. 1988). Since there is no database for the levels of 1,3-DNB and 1,3,5-TNB in human biological tissues or body fluids (other than in cases of accidental exposure), it is not possible to determine whether the existing analytical methods are sensitive enough to measure the background levels of the parent compound or metabolites in the general population or to measure the concentration levels at which biological effects occur in humans. Further testing and improvement of existing methods and development of new methods are needed for monitoring populations with potential for exposure to 1,3-DNB or 1,3,5-TNB.

Methemoglobinemia is a primary biomarker of effect for 1,3-DNB and 1,3,5-TNB. Well-established and reliable methods exist for monitoring methemoglobin formation using a complete blood count (Ishihara et a1.1976). However, methemoglobinemia is not a specific effect of 1,3-DNB and 1,3,5-TNB; other chemicals also cause methemoglobin formation. Other effects of exposure to 1,3-DNB and 1,3,5-TNB (cyanosis, headache, nausea, dizziness) are very general and cannot be quantified. Therefore, it would be useful to conduct further research to develop biomarkers of effect of exposure to 1,3-DNB and 1,3,5-TNB.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Several methods for determining 1,3-DNB and 1,3,5-TNB in environmental media have been developed and tested. In addition, some methods that have been developed for detection of nitrobenzene can also be used for detection of 1,3-DNB and 1,3,5-TNB (Cooper et al. 1986; Dangwal 1981; Kebbekus and Bozzelli 1982; Lorenzo et al. 1988). The methods expected to be most sensitive and selective for detecting the analytes in air are GC-based with detection by FID or NPD (Andersson et al. 1983; Cooper et al. 1986; Kebbekus and Bozzelli 1982). More data on these methods when used specifically to test for 1,3-DNB and 1,3,5-TNB are needed, because Andersson et al. (1983) provided no information regarding the detection limit of the GUFID method used for the determination of 1,3-DNB in air, and very few published methods are available that give that information. Both HPLC/UV and GC (high-resolution or low-resolution), combined with one of several detectors (NPD, ECD, FID, TEA, and MS), yield good results when water is analyzed for nitrobenzene compounds (Army 1983, 1986, 1989; Austem et al. 1975; EPA 1986a; Feltes and Levsen 1989; Feltes et al. 1990; Richard and Junk 1986; Stemmler and Hites 1987). Some of these methods have not been fully developed for analysis of 1,3-DNB and 1,3,5-TNB (Army 1983, 1989; Austem et al. 1975; EPA 1986a; Stemmler and Hites 1987). A detection limit of <0.1 μg/L and a

recovery of 74-89s has been reported for 1,3-DNB and 1,3,5-TNB in tap water by HRGC/ECD methodology (Richard and Junk 1986). Further testing of these methods specifically designed to determine their usefulness for measuring 1,3-DNB and 1,3,5-TNB in water would be helpful because no detection limit has been reported for the determination of these compounds in water by other promising methods, such as HPLC/UV/UV/PC (Army 1989) and GC/NPD following sample concentration by a suitable solid adsorbent during collection. The method currently used for detection of 1,3-DNB and 1,3,5-TNB in soil is reliable (Army 1985a; Jenkins et al. 1989), but increased sensitivity would allow better detection of trace levels in potentially contaminated soils. Since the background levels of 1,3-DNB and 1,3,5-TNB in ambient air, water and soil have not been established, it is not possible to determine whether the existing methods would be sensitive enough to measure 1,3-DNB and 1,3,5-TNB concentrations in background samples. Very little data were located on methods of analyzing for 1,3-DNB and 1,3,5-TNB in other media. The methods located for detection of the analytes in both beverages (Lorenzo et al. 1988) and explosives (McLuckey et al. 1985) were still in the developmental stages. Methods for analyzing these compounds in other media, especially in foods, plants, and aquatic and terrestrial organisms, are needed.

Some of the aerobic and anaerobic biodegradation products of 1,3-DNB in the environment are 1,3-nitroaniline, 1,3-phenylenediamine, 1,3-nitrophenol, 1,3-aminophenol and resorcinol (1,3-dihydroxyphenol) (Dey and Godbole 1986; Hallas and Alexander 1983; Reddy et al. 1993). A reversed-phase HPLWUV method is available for the determination of reduction products of 1,3-dinitrobenzene in the presence of 1,3-DNB and 1,3,5-TNB (Reddy et al. 1993). The derivatization of the amino-group with trifluoroacetic anhydride may increase the sensitivity of 1,3-nitroaniline and 1,3-phenylenediamine determination by the HPLCXJV method (Preslan et al. 1993). However, this method has not been standardized for the determination of reduction products of 1,3-DNB and 1,3,5-TNB.

6.3.2 Ongoing Studies

No ongoing studies regarding analytical methods were’ located for either 1,3-DNB or 1,3,5-TNB.