6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 2-nitrophenol and 4-nitrophenol 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 2-nitrophenol and 4-nitrophenol. 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 2-nitrophenol and 4-nitrophenol in environmental samples are the methods approved by federal agencies 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 refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

The analytical methods for determining nitrophenol in biological matrices are given in Table 6-1. The methods for handling biological samples are given by Fatiadi (1984). No promulgations concerning methods officially approved for use by federal or private trade groups could be located for 2-nitrophenol or 4-nitrophenol. All the methods presented in Table 6-l are for determining 4-nitrophenol in plasma or urine. Since 4-nitrophenol is a well known urinary metabolite in parathion-exposed subjects (Kirby et al. 1979), it is not surprising that the methods attempt to quantify 4-nitrophenol. However, there is no reason to believe that the methods applicable to 4-nitrophenol will not be applicable to 2-nitrophenol. The detection limit and accuracy will change for the two isomers for the same method (Section 6.2). The nitrophenols are not very volatile, and the determination of these compounds by GC usually requires derivatization to more volatile products. On the other hand, determination of these compounds by HPLC does not require derivatization. Among the commonly used methods, GC with electron capture detection of fluorinated derivates using heptafluorobutyric anhydride is probably the most sensitive method (Kirby et al. 1979). Other less commonly used methods, such as the Hall electroconductivity detector (HECD), dropping-mercury electrode, pulse polarography, and an enzymatic procedure are given by Fatiadi (1984) and Kirby et al. (1979).

4-Nitrophenol is excreted in urine entirely as glucuronide and sulfate conjugates (Fatiadi 1984). The sequential analysis of hydrolyzed (enzymatically or by acid) and unhydrolyzed urine provides a measure of the conjugated and unconjugated levels of the compound. Derivatization subsequent to acid hydrolysis of urine and quantification by gas chromatography with electron capture detection provides one of the most sensitive methods for 4-nitrophenol (Fatiadi 1984).

TABLE 6-1. Analytical Methods for Determining 2-Nitrophenol and 4-Nitrophenol in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Acid hydrolysis, extraction, derivatization, silica gel chromatography (for 4-NP)	GC-EC	20 μg/L	85-98	Shafik et al. 1973
Urine	Diluted in distilled water (4-NP and its conjugates)	HPLC-UV	no data	>98 (4-NP) >95 (conjugates)	Diamond and Quebbemann 1979
Plasma	Vortexed with methanol and supernatant concentrated (4-NP and its conjugates)	HPLC-UV	no data	>98 (4-NP) >95 (conjugates)	Diamond and Quebbemann 1979
Urine	4-Ethoxynitrobenzene obtained by method of Shafik et al. (1973) reduced and converted to amide by heptafluorobutyric anhydride (4-NP)	GC-EC	10 μg/L	No data	Kirby et al. 1979
Urine	Acid hydrolysis, extraction, and complexation with o-cresol in presence of $TiCl_3$ (4-NP)	spectrophotometric	30 μg in sample	92-100	Fatiadi 1984

GC-EC = gas chromatography-electron capture detection
HPLC-UV = high-pressure liquid chromatography-ultraviolet detection
4-NP = 4-nitrophenol

6.2 ENVIRONMENTAL SAMPLES

Analytical methods for determining 2-nitrophenol and 4-nitrophenol in environmental samples are given in Table 6-2. The handling methods for environmental samples are given in EPA, 1982. The nitrophenols probably exist predominantly in the vapor phase in the air (see Chapter 5.3.1), but small amounts of both compounds have been detected in the particulate phase (Leuenberger et al. 1985; Nojima et al. 1983). Therefore, the best method for collecting nitrophenols in air is to use an air sampler that uses glass-fiber filters to collect the particulate matter, followed by adsorption cartridges for trapping the volatile components (Leuenberger et al. 1985). Methods that are designed for multicomponent analysis use sample extraction with organic solvent(s) under both acidic and basic conditions. Nitrophenols, being acidic, are found in the acidic extract. In a recent evaluation of the EPA-approved method 625, a single continuous extraction at pH 2 was most efficient for determining both acidic and basic/neutral components in a sample. Additionally, the use of fused silica capillary columns may enhance both the efficiency and detection limits of various components, including nitrophenols, in multicomponent analytical methods such as the EPA method 625 (Valkenburg et al. 1989).

Among the commonly used methods, GC with electron capture detection of the heptafluorobutyryl derivative provides the greatest sensitivity. However, the GC-MS method has the most versatility and is more suitable where multicomponent analysis is required. Several other less commonly used methods are available for determining 2-nitrophenol and 4-nitrophenol in environmental samples. Some of these methods are spectrometric measurement in the presence of crown ethers (Papadoyannis et al. 1983), coulometric measurement with methylviologen radical cation (Lozano et al. 1989), HPLC-surface-enhanced resonance Raman scattering (Ni et al. 1989), GC-FID (flame ionization detector) with a special graphitized carbon black as the GC stationary phase (Mangani et al. 1986), remote fluorescence analysis of ground water with UV lasers and fiber optics (Chudyk et al. 1985), HPLC with diode-array UV-visible - detector (Nielen et al. 1985), and cyclic voltammetric determination by the addition of a-cyclodextrin (Matsue et al. 1981).

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-nitrophenol and 4-nitrophenol is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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-nitrophenol and 4-nitrophenol.

TABLE 6-2. Analytical Methods for Determining 2-Nitrophenol and 4-Nitrophenol in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Thermal desorption of cartidge	FSCC-MS/DS	No data	No data	Leuenberger et al. 1985
Air	Extract filter, clean extract, treated with diazomethane and concentrated	GC-MS	No data	No data	Nojima et al. 1983
Waste water	Extract, clean extract, derivatized with pentafluoro-benzyl bromide	GC-EC (EPA Method 604)	0.77 µg/L (2-NP) 0.70 µg/L (4-NP)	67 (2-NP) 45 (4-NP)	EPA 1982
Water	Extract, concentrated and mixed with hexadecyltrimethyl-ammonium bromide and K_2CO_3	HPLC-UV	1 µg/L (4-NP)	81-88	Roseboom et al. 1981
Water	Resin sorption, desorption, and concentration	HPLC-UV	0.18 µg/L (for 10 mL)	97.4-105.7	Borys 1981
Water	Sample reacted with iodine monobromide, extract	Absorbance at 240 nm (4-nitrophenol)	3 µg/L	No data	Bosch et al. 1987
Water	Extract, derivatized with heptafluorobutyryl anhydride and concentrate	GC-EC	0.01 µg/L (2-NP) 0.01 µg/L (4-NP)	73 (2-NP) 40-43 (4-NP)	Bengtsson 1985
Water	Extract, clean extract, concentrate	GC-MS/DS	1 µg/L (2-NP) 5 µg/L (4-NP)	No data	Sporstoel et al. 1985
Water, waste water	Extract, concentrate	GC-MS (EPA Method 625)	3.6 µg/L (2-NP) 2.4 µg/L (4-NP)	75 both in water and waste water (for 2-NP) 41 in water and 43 in waste water (for 4-NP)	EPA 1982
Sediment/soil	Extract, concentrate, and clean-up	GC-MS (EPA CLP method)	330 µg/kg (2-NP) 1600 µg/kg (4-NP)	No data	EPA 1988b

CLP = contract laboratory program; EC = electron capture detection; FSCC-MS/DS = fused silica capillary, mass spectrometry/data system; GC = gas chromatography; HPLC-UV = high-resolution liquid chromatography - ultraviolet detection; 2-NP = 2-nitrophenol; 4-NP = 4-nitrophenol

6. ANALYTICAL METHODS

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. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No biomarker that can be associated quantitatively with exposure to 2-nitrophenol or 4-nitrophenol has been identified (see Section 2.5). If a biomarker for these compounds in a human tissue or fluid were available and a correlation were found to exist between the level of biomarker and exposure/health effect, the biomarker could be used as an indication of health effects caused by the exposure of these chemicals.

No specific effects of 2-nitrophenol or 4-nitrophenol exposure have yet been identified (see Section 2.5.2).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods with good sensitivity and specificity for determining the two compounds in contaminated water and soil are available (see Table 6-2). Dr. Milton Lee of Brigham Young University has recently developed an analytical method for the quantification of femtogram quantities of nitrophenols in air using time-of-flight mass spectrometer (Sin et al. 1991). Besides this method, analytical methods for the determination of low levels of nitrophenols found in ambient air and data on the accuracies, precisions, and sensitivities of such methods are lacking. The levels of these two compounds in drinking water have very rarely been measured. It is not clear whether this limitation in data is due to lack of effort directed to measure the levels, lack of method sensitivity, or the presence of these compounds at extremely low levels. Analytical methods are available for determining most of the final biodegradation and photodegradation products of these compounds (Raymond and Alexander 1971; Sethunathan 1973; Zeyer and Kearney 1984). However, the accuracy and precision of these methods have rarely been established. The intermediate products remain unknown or unidentified in many cases. The levels of the parent compounds in different environmental media can be used to indicate exposure to these compounds by humans through the inhalation of air and ingestion of foods and drinking water, when the typical volume of air inhaled and drinking water consumed daily and the daily average amount and composition of adult total diet samples are known (Gartell et al. 1986). If a correlation between the levels of these compounds in human tissue and the levels of exposure could be found, the exposure levels from different environmental sources could be used to estimate human body burden. Similarly, determining degradation products is important because it may assist in the need for evaluating the toxicity of the products

and determining the persistence of the parent compound. In instances where the products of an environmental reaction are more toxic than the parent compound, it is important that the level of the degradation products in the environment be known.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of 2-nitrophenol and 4-nitrophenol and other phenolic compounds in urine. These methods use high resolution gas chromatography and magnetic sector mass spectrometry which gives detection limits in the low parts per billion range.

No other on-going studies pertaining to the determination of the nitrophenols in biological or environmental media were found.