6. ANALYTICAL METHODS

6.1 BIOLOGICAL MATERIALS

Methods used for the analysis of N-nitrosodi-n-propylamine in biological samples are shown in Table 6-l. The use of vacuum distillation at low temperature (60-70°C) and trapping the distillate in dry ice/acetone bath is the preferred method for the isolation of the compound from biological matrices. Because of its selectivity and sensitivity, methods using Thermal Energy Analyzer (TEA) detectors are selected for quantification of this compound. In cases where analysis of multiple pollutants are necessary, mass spectrometric detectors, in spite of their lower sensitivity for nitrosodi-n-propylamine, may be favored over the TEA detector.

6.2 ENVIRONMENTAL SAMPLES

Methods used for the analysis of N-nitrosodi-n-propylamine in environmental samples are given in Table 6-2. Two pretreatment methods are most suitable for the analysis of this compound in environmental samples. When the matrix is not too complex, as in the case of drinking water, surface water, groundwater and wastewater, solvent extraction is the preferred method. With more complex matrices as soil and foodstuffs, vacuum distillation and cold trapping is more suitable. The three methods commonly used for the quantification of this compound in environmental samples are mass spectrometry, nitrogen-phosphorus detectors (NPD) and TEA in chemilumin escence mode. Each of these detectors has its own advantages and disadvantages. When a high sensitivity is required, a method using a TEA detector may be the method of choice. For multipollutant analysis in a single sample, mass spectrometry may be more suitable. Nitrogen-phosphorus detectors, on the other hand, may be more cost-effective and will provide reasonable specificity and sensitivity for nitroso compounds. A Hall detector in the pyrolytic mode may be preferable over NPD detectors because it may require less sample clean-up (Rhoades et al. 1980).

6.3 ADEQUACY OF THE DATA BASE

Section 104 (i) (5) of CERCLA, 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 N-nitrosodi-n-propylamine is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects (and techniques for developing methods to determine such health effects). The following discussion highlights the availability, or absence, of exposure and toxicity information applicable to human health assessment. A statement of the

TABLE 6-1. Analytical Methods for N-Nitrosodi-n-propylamine in Biological Samples

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Urine	Extracted with solvent, cleaned by washing with water, extract dried, concentrated, and subjected to acid catalyzed denitrosation and reaction of evolved NO with ozone.	Chemiluminescence detection	0.05 µg/L (for 100 mL sample)	65% at 11 ppb	Drescher and Frank 1978
Postmortem tissues (brain, liver, kidneys, and pancreas)	Organs mixed with KOH and ammonium sulfamate homogenized, vacuum-distilled distillate solvent extracted and concentrated. Concentrate derivatized for ECD only.	GC-ECD and GC-TEA	<0.5 µg/kg (for 50 g sample)	77-88% (GC-ECD)	Cooper et al. 1987
Liver, kidney, and blood	Organs mixed with sulfuric acid and ammonium sulfamate homogenized and extracted with solvent and concentrated.	GC-TEA	0.6 ng/kg	91% (liver) 96% (kidney) 83% (blood)	Maki 1980
Urine, blood, feces, saliva and gastric contents	Homogenized sample mixed with paraffin or glycerol, water and sodium hydroxide, vacuum distilled at low temperature. Distillate extracted on-column or by shaking with solvent and extract concentrated.	GC-TEA	0.05-0.5 µg/kg	79% (for urine)	Eisenbrand et al. 1983

GC = Gas chromatography; ECD = electron capture detected; TEA = thermal energy analyzer

TABLE 6-2. Analytical Methods for N-Nitrosodi-n-propylamine in Environmental Samples

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Occupational air	Sorbed onto ThermoSorb/N, desorbed by CH_2Cl_2/CH_3IG	HRGC-MS	<0.1 $\mu g/m^3$ (monitoring NO^+) <0.04 $\mu g/m^3$ (monitoring parent ion)	NG	Cooper 1987
Water and wastewater	Solvent extracted, acid washed, column chromatographic cleanup	GC-NPD; GC-reductive HECD GC-TEA (EPA Method 607))	0.46 µg/L (GC-NPD)	61% at 9 µg/L	EPA 1982
	Solvent extracted, acid washed, column chromatographic cleanup	GC-NPD (collaborative study on EPA Method 607)	0.46 µg/L (clean water) 0.36-0.74 µg/L (wastewater)	82% at 1.22 µg/L 84% at 26.7 µg/L 92% at 1.22 µg/L 94% at 26.7 µg/L	Millar et al. 1984
	Solvent extraction under basic condition, solvent concentrated	GC-MS (EPA Method 625)	NG	68% (reagent water) 76% (wastewater)	EPA 1982
Water	Solvent extracted, water washed and concentrated, acid catalyzed denitrosation and reaction evolved NO with ozone	Chemiluminescence detection	0.05 µg/L (for 100 mL sample)	61% at 11 ppb (11 µg/L)	Drescher and Frank 1978
	Direct injection	HPLC separation, resolved nitrosoamine photolyzed to nitrite and detected colorimetrically	NG	NG (suggested screening method)	MacMillan 1983
Waste and wastewater	Solvent extraction, column chromatographic cleanup, if necessary, and concentration	GC-AFID (nitrogen mode) GC-Hall (pyrolytic mode) GC-TEA	NG	54-103% (AFID) 73-108% (Hall) 78-104% (TEA) at 1.2 µg/L	Rhoades et al. 1980
	Solvent extraction under neutral condition, solvent concentrated	HRGC-MS (EPA Method 625.1)	<10 µg/L	67% at 20 µg/L	Eichelberger et al. 1983
Groundwater	Solvent extraction at pH 11, solvent concentrated	GC-MS (EPA CLP Method)	10 µg/L	NG	EPA 1987, Fisk 1986

TABLE 6-2 (continued)

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Groundwater	Solvent extraction, column chromatographic cleanup, concentration of extract	GC-MS (EPA Method 8250)	NG	79% at 19 µg/L	EPA 1986b
	Solvent extraction, column chromatographic cleanup, concentration of extract	HRGC-MS (EPA Method 8270)	10 µg/L	79% at 19 µg/L	EPA 1986b
Soil	Aqueous extract subjected to combined distillation and solvent extraction, and extract concentrated	GC-FID	0.025 µg/g	81-87%	Pancholy 1976
	Homogenized sample mixed with paraffin or glycerol, water and NaOH, vacuum distilled at low temperature. Distillate extracted on-column or by shaking with solvent and extract concentrated	GC-TEA	0.05-0.5 µg/g	71%	Eisenbrand et al. 1983
	Sample solvent extracted, washed with water and concentrated concentrate, subjected to acid catalyzed nitrosation and evolved NO reacted with ozone	Chemiluminescence detection	0.05 µg/kg	69% at 11 ppb (11 µg/kg)	Drescher and Frank 1978
Soil/sediment	Solvent extracted, column chromatographic cleanup if necessary, extract concentrated	GC-MS (EPA CLP Method)	330 µg/kg	NG	EPA 1987, Fisk 1986
Soil, sludge or solid waste	Solvent extracted by Soxhlet or or sonication, extract subjected to column chromatographic cleanup, concentration of extract	GC-MS (EPA Method 8250)	NG	NG	EPA 1986b
Soil, sludge or solid waste	Solvent extracted by Soxhlet or or sonication, extract cleaned by column chromatography and concencentrated	HRGC-MS (EPA Method 8270)	660 µg/kg	NG	EPA 1986b
Whiskey and beer	Sample mixed with paraffin or glycerol, water and NaOH vacuum distilled at low temperature. Distillate solvent extracted and concentrated	GC-TEA	0.05-0.5 µg/L	77-86%	Eisenbrand et al. 1983

TABLE 6-2 (continued)

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Beans and fish	Blended sample distilled by freeze drying, extracted in solvent and concentrated	GC-FID	3 µg/g	90.6-99.4%	Du Plessis and Nunn 1972
Cooked bacon	Alkaline sample vacuum distilled, trapped in liquid N_2, extracted with solvent and concentrated	GC-TEA	NG	82% at 10 µg/kg	Greenfield et al. 1982
Canned tuna, corned beef, soya bean oil, deep fried fish, and french fries	Vacuum distillation from mineral oil extraction with solvent and concentrated	GC-TEA	5 µg/kg	95-100%	Fine et al. 1975
Rubber nipple	Sliced sample extracted with solvent and extract concentrated	HPLC-TEA with a cold trap	NG	NG	Rühl and Reusch 1985
	Sliced sample solvent extracted, solution made alkaline and distilled. Aqueous distillate solvent extracted and concentrated	GC-TEA with a cold trap	NG	10-120%	Gray and Stachiw 1987
Cosmetic products	Sample mixed with aqueous ammonium sulfamate and cleaned with $CHCl_3$. The aqueous solution cleaned up by a HPLC pre column	HPLC-DPP	0.2 ppb	NG	Vohra and Harrington 1981

NG = Not given; HRGC = high resolution gas chromatography, MS = mass spectrometry; NPD = nitrogen-phosphorus detector, HECD = Hall electrolytic conductivity detector, TEA = thermal energy analyzer; GC = gas chromatography; HPLC = high presure liquid chromatogaphy; AFID = alkali flame ionization detector; FID = flame ionization detector; DPP = differential pulse polanography

relevance of identified data needs is also included. In a separate effort, ATSDR, in collaboration with NTP and EPA, will prioritize data needs across chemicals that have been profiled.

6.3.1 Data Needs

Methods for Determining Parent Compound and Metabolites in Biological Materials. Analytical methods of suitable sensitivity and specificity are available for the quantification of N-nitrosodi-n-propylamine in biological samples. The study of the levels of the parent compound in human blood, urine or other biological matrices can be useful in deriving a correlation between the level of this compound found in the environment and those found in human tissue and body fluid. Such correlation studies are not available for N-nitrosodi-n-propylamine. The changes in metabolite concentrations with time in human blood, urine, or other appropriate biological medium may be useful in estimating its rate of metabolism in humans. In some instances, a metabolite may be useful in correlating the exposed doses to the human body burden. Such studies on the levels of metabolites in human biological matrices are not available for this compound, although metabolic products of this compound from animal and in vitro studies have been identified (see Subsection 2.6.3) and analytical methods for their quantification are available.

Methods for Biomarkers of Exposure. No known biomarker for exposure to N-nitrosodi-n-propylamine has been identified (see Subsection 2.9.2). 7-Methyldeoxyguanosine and 06-methyldeoxyguanosine adducts are formed as a result of exposure to methylated nitrosoamines. Immunological assays are available for the determination of these adducts (Degan et al. 1988, Foiles et al. 1988). If propyl-substituted deoxyguanosine adducts are formed from N-nitrosodi-n-propylamine by similar reaction, it could be used as a biomarker for N-nitrosodi-n-propylamine exposure. If such a biomarker were available and a correlation were found between the level of the biomarker and exposure, it could be used as a measure of exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods of suitable sensitivity and specificity are available for the quantification of this compound in environmental samples. The levels of this compound in different environmental media can be used to indicate exposure of humans to this compound through the inhalation of air and ingestion of drinking water and foods containing N-nitrosodi-n-propylamine. If a correlation with human tissue or body fluid levels were available, the intake levels from different environmental sources could be used to estimate the body burden of the chemical in humans. Although the products of biotic and abiotic processes of this compound in the environment are adequately known, no systemic study is available that measured the concentrations of its degradation products in the environment. In instances where the product(s) of an environmental reaction is more toxic than the parent compound, it is important that the level of the degradation products in the environment be known. However, the

degradation products of this compound in environmental media are less toxic compounds, namely n-propylamine, di-n-propylamine etc. (see Subsection 5.3.2). The analytical methods for the determination of the levels of these and other environmental degradation products of N-nitrosodi-n-propylamine are available.

6.3.2 On-going Studies

No significant on-going studies are in progress for the development of analytical methodologies for this compound in environmental or biological samples.