6. ANALYTICAL METHODS

6.1 BIOLOGICAL MATERIALS

Methods used for the quantification of NDMA in biological samples are given in Table 6-1. Two problems encountered in the analysis of NDMA are poor recovery of the compound due to its high volatility and the artifactual formation of this compound during sample storage and treatment (Fine 1982). Since nitroso compounds are formed in acid solution, keeping the solution alkaline during storage and treatment may reduce artifact formation (Kosaka et al. 1984). Other authors have used ascorbic acid to inhibit in vitro formation of nitrosamines and have used morpholine to measure the extent of in vitro nitrosation during storage and handling (Dunn et al. 1986).

The method that has most selectivity for the quantification of this compound is thermal energy analyzer (TEA). A few investigators have oxidized this compound with pentafluoroperoxybenzoic acid to achieve higher sensitivity with electron capture detector (ECD).than with TEA (Kimoto et al. 1984). However, ECD detectors have less selectivity than TEA and will require more sample clean up. The confirmation of NDMA in a sample is usually done by mass spectrometry (MS). Samples containing small amounts of NDMA cannot be detected by MS in the presence of large background impurities (as in samples treated for TEA analysis). Photolysis at 366 nm affords an alternative means for validating the presence of this compound identified by TEA (Cooper et al. 1987). A method for the analysis of total N-nitroso compounds in gastric juice is also available (Pignatelli et al, 1987).

6.2 ENVIRONMENTAL SAMPLES

Methods for quantifying NDMA in environmental samples are summarized in Table 6-2. As with the biological samples, in situ artifact formation must be avoided in order to get accurate results from the analysis of environmental samples (Fisher et al. 1977; Fine et al. 1977a). The three quantification methods that give satisfactory sensitivity for NDMA are alkali flame ionization detector (in the nitrogen mode) (AFID), Hall electrolytic conductivity detector (HECD) in the reductive mode and TEA. The advantages and disadvantages of these detectors have been evaluated (Rhoades et al. 1980; Usero et al. 1987). Of the three detectors, the TEA detector has the highest sensitivity and selectivity. Because of its higher selectivity, the TEA detector cannot be versatile enough for multipollutant analysis. Mass spectrometric detector can be used not only for confirmation of the presence of NDMA in a sample, but for quantification as well (Eichelberger et al, 1983; Webb et al, 1979). When used in combination with a high resolution GC column, this method has the ability to quantify a large number of pollutants in a sample. The use of selected ion monitoring (SIM) may increase the sensitivity by orders of magnitude. The SIM method does not provide the full mass spectra necessary for the identification of unexpected compounds, however (Bellar et al. 1979). A method for the

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

Sample Matrix	Sample Preparation	Analytical Method	Detection	Accuracy Limit	Reference
Whole blood	Distill alkaline solution, extract distillate in solvent and concentrate.	GC-TEA	0.1 µg/L	95%	Lakritz et al. 1980
Blood, liver, kidney, brain	Sample with added sulfamic acid and antifoaming agent, subjected to simultaneous distillation and extraction, extract concentrated.	GC-TEA	<1 ppb	93-97% at 2.3-4.2 ppb	Pylypiw et al. 1985; Pylypiw 1987
Blood	Sample mixed with ascorbic acid and morpholine, subjected to distillation in alkaline solution, distillate extracted with solvent and concentrated.	GC-TEA	8 pg or 0.05 µg/kg (for 20 g sample)	93%	Dunn et al. 1986
	Alkaline sample dialyzed with solvent, dialyzates separated and concentrated.	HRGC-MS	3-4 pg	60-70%	Kosaka et al. 1984
	Vacuum distillation in mineral oil, extracted with solvent and concentrated.	GC-TEA	0.1 µg/L	NG	Gough et al. 1983
Brain, liver, kidney, pancreas	Sample mixed with ammonium sulfamate, homogenized and distilled under vacuum, extracted with solvent, derivatized with pertrifluoroacetic acid, cleaned by column chromatography and concentrated.	GC-TEA and GC-ECD	NG	54.7%	Cooper et al. 1987
Urine	Sample buffered at pH 10 extracted with solvent, solvent concentrated.	GC-HRMS	5 ng/L	99-103% at 10-80 ng/L	Garland et al. 1986

NG = Not given; GC = gas chromatography; TEA = thermal energy analyses; HRGC = high resolution gas chromatography; MS = mass spectrometry; ECD = electron capture detector; HRMS = high resolution mass spectrometry

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

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Ambient air	Sample collected in impinger containing KOH, extracted in solvent and concentrated.	GC-MS	10 pg	NG	Fisher et al. 1977
	Sample sorbed on Tenax, thermally desorbed.	Cryofocusing HRGC-MS	0.5 ppt (for 150 L air)	90-110%	Sawicki et al. 1977
	Collected in Tenax, thermally desorbed and trapped in a cryogenically cooled trap and dissolved in solvent and concentrated.	HRGC-MS	0.3 pg	NG	Webb et al. 1979
	Collected in ambient or cold KOH trap, extracted in solvent and concentrated.	GC-TEA	1 ng/m	43.6%	Fine et al. 1977a,b
Water, wastewater	Sample extracted with solvent, column chromatographic clean-up, concentration.	GC-NPD or GC-reductive HECD or GC-TEA (EPA Method 607)	0.15 µg/L	32% at 0.8 µg/L	EPA 1982
	Extract with solvent at pH 7, concentrate extract.	Cryofocusing HRGC-MS (EPA Method 625.1)	1-10 µg/L	42% at 100 µg/L	Eichelberger et al. 1983
Water	Extract with solvent, concentrate extract.	GC-TEA	2 ng/L	68%	Fine et al. 1977a,b
Water, wastewater	Extracted with solvent, column chromatographic clean-up if required, concentration of extract.	GC-AFID GC-TEA GC-reduction HECD	NG	32% (AFID) 42% (TEA) 43% (HECD)	Rhoades et al. 1980
Soil	Extracted with water, water extracted with solvent and concentrated.	GC-TEA	NG	NG	Fine et al. 1977b
Minced fish and surimi	sample eluted with solvent, cleaned by column chromatography and concentrated	GC-TEA	0.2 ppb	77-97% at 10 ppb	Pensabene and Fiddler 1988

TABLE 6-2 (continued)

Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy	Reference
Meat, vegetable	Vacuum distill ground sample, extract distillate with solvent, clean up by column chromatography, derivatize with peroxytrifluoroacetic acid and column chromatographic clean up and concentrate.	GC-ECD	0.2 ppb (for 250 g sample)	<78%	Telling 1972
Malt, beer, milk powder, cured meat	Clean sample by dry column/elution or mineral oil distillation method, clean up further by column chromatography, oxidize with pentafluoroperoxybenzoic acid, clean up by column chromatography and concentrate.	GC-TEA or GC-ECD	<1 ppb	NG	Kimoto et al. 1984
Malt beverages	Clean sample by celite column chromatography, concentrate methylene chloride eluate.	GC-TEA	NG	90%	Hotchkiss et al. 1981
Beer	Sample treated with sulfamic acid, distillation under basic condition, extraction with solvent and concentration.	GC-TEA	0.1 ppb	78-112% at 0.08-4 ppb	Sen and Seaman 1981a
Fried bacon	Clean sample by acidic celite column	GC-TEA	NG	101% at 10 ppb	Pensabene et al. 1982

GC = Gas chromatography; MS = mass spectrometry; HRGC = High resolution gas chromatography; TEA = thermal energy analyzer; NPD = nitrogen-phosphorus detector; HECD = Hall electrolytic conductivity detector; AFID = alkali flame ionization detector; ECD = electron capture detector; NG = not given

analysis of apparent total N-nitroso compounds in beer is also available (Massey et al. 1987).

6.3 ADEQUACY OF THE DATABASE

Section 104 (i) (5) of CERCIA, 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 NDMA 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 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 Compounds and Metabolites in Biological Materials. NDMA is equally distributed in the cellular elements of the blood and in the plasma and serum (Lakritz et al. 1980). Therefore, it is advantageous to analyze whole blood for the quantification of NDMA. Because NDMA is metabolized almost quantitatively in humans (Spiegelhalder 1984), determination of this compound in human urine needs an extremely sensitive technique (Garland et al. 1986). The urinary excretion of NDMA has been correlated with the concentration of NO_2 in air, suggesting that ambient air may play a role in the exposure of people to nitrosoamines (Garland et al. 1986). There is a paucity of data on the analytical methods for the determination of N-nitrosodimethylamine in human urine.

No metabolite of NDMA from human exposure to this compound has yet been identified (see Subsection 2.6.3). A metabolite identified in laboratory animal has been discussed in Subsection 2.6.3. 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.

Methods for Biomarkers of Exposure Recently, a radioimmunoassay was used to detect elevated levels of the promutagenic lesion 06-methyldeoxyguanosine in DNA cells from individuals with high incidence of cancer who consumed foods with a high nitrosamine content (Wild et al, 1987). Although no correlation has been established between the DNA-adduct

and the level of NDMA in consumed foods, the DNA-adduct has the potential to be used as a biomarker for exposure to NDMA.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The levels of this compound in 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-nitrosodimethylamine. 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. Such studies correlating the levels of this compound in any environmental medium with the levels in any human tissue or body fluid are not available.

Although the products of biotic and abiotic processes of this compound in the environment are known, no systematic study is available that measured the concentrations of its reaction products in the environment. In instances where the products of an environmental reaction are more toxic than the parent compound, it is important that the level of the reaction products in the environment be known. N-nitrosodimethylamine is not likely to form more toxic products as a result of environmental reactions (see Subsection 5.3.2). The analytical methods for the determination of the levels of environmental reaction products of N-nitrosodimethylamine are available.

6.3.2 On-going Studies

No ongoing studies are in progress for the improvement of the analytical method for NDMA in biological samples. Studies are currently conducted by J. Conboy and J. Hotchkiss at Cornell University, Ithaca, NY and by D. Havery at FDA, Washington, DC, for the development of analytical methods for this compound in environmental samples.