VOLATILE NITROSAMINE MIXTURE I
N-NITROSODIMETHYLAMINE (NDMA)
N-NITROSODIETHYLAMINE (NDEA)
N-NITROSODI-n-PROPYLAMINE (NDPA)
N-NITROSODI-n-BUTYLAMINE (NDBA)
N-NITROSOPIPERIDINE (NPIP)
N-NITROSOPYRROLIDINE (NPYR)
N-NITROSOMORPHOLINE (NMOR)

Method no: 27

OSHA PEL: The OSHA standard applies to all solid or liquid mixtures containing more than 1% NDMA by weight or volume.

Procedure: Samples are collected using ThermoSorb/N air samplers. The samples are desorbed with a 75/25 (v/v) dichloromethane/methanol solution. Analysis is performed by gas chromatography with Thermal Energy Analyzer detection.

Recommended air volume and sampling rate: 75 L at 1 L/min (0.2 to 2 L/min is permissible)

Volatile Nitrosamine Mixture I

analyte		NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
target concn	ppb	0.89	0.65	0.51	0.37	0.51	0.66	0.84
	$\mu g/m^3$	2.7	2.7	2.7	2.4	2.4	2.7	4.0
reliable quantitation limit	ppb	0.043	0.031	0.024	0.019	0.026	0.032	0.042
	$\mu g/m^3$	0.13	0.13	0.13	0.12	0.12	0.13	0.20
standard error of estimate at target concn, (Section 4.7)	%	5.81	6.24	6.22	6.26	5.74	5.86	6.28

Special requirements: It is recommended that samples be stored in a freezer.

Status of method: A sampling and analytical method that has been subjected to the established procedures of the Organic Methods Evaluation Branch.

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1. General

1.1 Background

1.1.1 History

The purpose of this work was to develop a general sampling and analytical procedure for volatile N-nitrosamines. This method is intended to supersede previous OSHA methods which were each evaluated for only one N-nitrosamine. This method is superior to previous methods because it permits the industrial hygienist to monitor seven different N-nitrosamines with a single sample.

Volatile N-nitrosamines have been collected using successive cold traps (-79 to -95°C) in series (Ref. 5.1), ambient temperature KOH bubblers (Ref. 5.2), pH 4.5 ascorbic acid bubblers (Ref. 5.3) and solid sorbents which include Tenax GC (Ref. 5.4), activated charcoal-water suspension (Ref. 5.5), vitamin E and C treated Florisil adsorbent tubes (Refs. 5.6 and 5.7), XAD-4 resin (Ref. 5.8) and ThermoSorb/N commercial nitrosamine air samplers (Ref. 5.9). Activated alumina, silica gel and Florisil adsorbents have been tested, but not widely used to sample air for nitrosamines (Ref. 5.9).

Many of these air sampling techniques have serious deficiencies. The cold traps are difficult to maintain and are reported to enable the artifactual formation of NDMA from precursor amines and nitrogen oxides (Ref. 5.10). The ambient temperature KOH and ascorbic acid bubblers are free of artifact formation but analyte retention efficiency decreases with increasing sampling time and temperature (Ref. 5.9). Tenax GC has a relatively low breakthrough volume for NDMA (Ref. 5.9). Solid adsorbents, in general, may concentrate precursor amines and nitrosating agents resulting in artifactual in situ formation of the analyte (Ref. 5.9). The vitamin E and C treated Florisil adsorbent tubes were shown to be artifact resistant, but no one device was adequate for use as a general-purpose nitrosamine air sampler (Ref. 5.7).

Because of the many problems associated with alternative air sampling techniques, it was decided to evaluate the ThermoSorb/N air sampling system. The device is a commercially available opaque plastic cartridge containing a solid adsorbent. The manufacturer states that the device is designed to prevent the artifactual formation of the analytes because of a proprietary amine trap and nitrosation inhibitor.

Almost all analytical methods for N-nitrosamines utilize some sort of chromatographic separation procedure prior to detection of the analyte. Some samples, which usually have a simple matrix, have been determined directly using polarographic and spectrophotometric methods. These techniques are subject to interferences and have limited application for low level analysis of complex samples (Ref. 5.11).

Thin layer chromatographic (TLC) techniques have been used to determine N-nitrosamines. The analytes have been chromatographed before and after derivatization. The derivatives include nitramines, fluorescent dansyl products, fluorescent hydrazones and hydrazines. The underivatized analyte has been visualized using sprays that form a colored complex with the parent secondary amine obtained by exposing the chromatographed N-nitrosamine to ultraviolet (UV) light. The main advantages of TLC methods are that they are simple, inexpensive and some have very low detection limits. The main disadvantages are that they are only semiquantitative and do not have sufficient resolution (Ref. 5.11).

High performance liquid chromatographic (HPLC) techniques have been used to determine complex mixtures of N-nitrosamines. The analytes have been chromatographed and detected as N-nitrosamines and as 2,4-dinitrophenyl derivatives of the parent secondary amine. The UV detector is usually employed in these methods. Advantages of HPLC procedures include the capability to chromatograph non-volatile and thermally labile analytes. The main disadvantage has been the lack, until recently, of selective detectors (Ref. 5.11).

The most widely used technique to separate complex mixtures of volatile N-nitrosamines is gas chromatography (GC). Volatile derivatives of non-volatile N-nitrosamines have been prepared for GC analysis also. Volatile N-nitrosamines chromatograph quite well on stainless steel columns and there is no apparent advantage to using glass GC columns. The GC column packing most used is 10% Carbowax 20M with 2% KOH on 80/100 mesh

acid washed Chromosorb W. Other stationary phases that have been used are Reoplex 400, FFAP, Carbowax 1540, BDS and Versamid. Capillary and SCOT columns have been used and very good peak resolution has been obtained (Ref. 5.11).

Most of the commonly available GC detectors have been used to determine N-nitrosamines. These detectors include the flame-ionization detector, nitrogen selective alkali flame-ionization detector, Coulson Electrolytic Conductivity Detector and Hall Electrolytic Conductivity Detector. Derivatives that are sensitive to electron capture detectors have been prepared from N-nitrosamines. The main disadvantage to these detectors is that they are not selective for N-nitrosamines. Modified Hall and Coulson Electrolytic Conductivity detectors have been used to increase selectivity to N-nitrosamines.

The Hall detector is very similar in principle to the Coulson detector. In the normal operation of these detectors, the GC column effluent is mixed with hydrogen gas and passed through a heated quartz reaction tube containing a nickel catalyst. The nitrogen containing analyte is reduced to ammonia which is measured by a change in the conductivity of water in the detector cell. Acidic interferences are removed with a scrubber. The catalyst is removed to modify the detectors and the modified detectors respond only to N-nitrosamines and amines (Ref. 5.11).

Since mass spectrometry provides an unequivocal means to confirm chemical structure, it has been used as a GC detector in the determination of complex mixtures containing N-nitrosamines (Ref. 5.4).

The Thermal Energy Analyzer (TEA) is a highly selective detector for N-nitroso compounds that has been successfully interfaced to gas and liquid chromatographs. In the GC mode of operation, the chromatographed N-nitroso compound exits the GC and enters the TEA pyrolyzer through a heated transfer line. The chemical bond between the two nitrogen atoms (N-NO) is thermally broken, resulting in an organic fragment and a nitrosyl radical (·NO). Organic compounds, solvents and fragmentation products are collected in a cold trap. The nitrosyl radical is a stable gas which passes through the cold trap to react with ozone under vacuum to form electronically excited nitrogen dioxide. The excited nitrogen dioxide quickly decays to its ground state and emits light, at a characteristic wavelength, which is measured by a photomultiplier tube. When the TEA is used as an HPLC detector, the sequence of events is similar to those of the GC mode. The chromatographed analyte exits the HPLC column and enters the TEA pyrolyzer. A higher temperature pyrolyzer is used because the HPLC mobile phase is flashed at the same time the N-nitroso bond is thermally broken. The vaporized HPLC mobile phase, organic compounds and fragmentation products are condensed in a cold trap. The nitrosyl radical is swept through the cold trap with an inert gas, usually helium or argon, and is detected as before (Ref. 5.48).

This method recommends separation by GC and detection with the TEA. An HPLC method is also presented for use as a confirmatory procedure.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy).

Detailed toxicology information regarding chronic exposure to the individual analytes is presented in Section 4.8. Much of the toxicology data regarding the analytes have been obtained using rats as the test species and that work is presented in this method. Some of the analytes have been investigated using other species and similar toxic effects have been observed.

The effects of acute exposure to each of the analytes were similar. The administration of a lethal dose to rats led to a gradually advancing weakened, emaciated condition frequently with the appearance of jaundice. Death usually occurred within seven days. The autopsy revealed severe centrilobular liver damage with hemorrhaging into the lungs in most cases. The response to a lethal dose was the same for male and female rats (Ref. 5.12).

Chronic exposure to the analytes has led to cancer in a number of different animal species (Ref. 5.15). The carcinogenic potential of the analytes varies over a wide range and depends on the compound, route of administration, level of dose, frequency of dose and length of the exposure.

N-nitrosodialkylamines do not appear to be directly active carcinogens. If the compounds were directly active, local sarcomas should be observed at the site of subcutaneous injection because of the high concentration of the agent and sensitivity of the subcutaneous tissue. If, however, local sarcomas do not develop and tumors in distant organs are observed, the agent is an intermediate from which the ultimate carcinogen is produced by metabolic processes. Subcutaneous injection of N-nitrosodialkylamines did not result in local sarcomas and the carcinogenic effects were evident in specific organs (lungs, liver, bladder, etc.). The N-nitrosodialkylamines are, therefore, considered not to be the ultimate carcinogens (Ref. 5.12).

The carcinogenic effects of dialkylnitrosamines on the molecular level, is thought to be the result of an alkylation of nucleic acids. The metabolic activation of a N-nitrosodialkylamine involves the enzymatic hydroxylation of the carbon atom immediately adjacent (in the alpha position) to the N-nitroso group. Several intermediate species are formed, one of which is diazoalkane. The ultimate carcinogen is believed to be a carbonium ion. A schematic of the proposed biological activation process is presented in Figure 1.1.2. The theory is supported by the fact that exposure of rats to ethyl-tert-butylnitrosamine, which cannot form a diazoalkane, was not carcinogenic, even in high doses. Exposure to ethyl-n-butylnitrosamine, however, did lead to cancer in rats (Ref. 5.12).

The mechanism by which the cyclic N-nitrosamines (NPIP, NPYR, NMOR, etc.) exert their carcinogenic action is not as clear as that for the dialkylnitrosamines. It is unknown if the rings contained in these cyclic compounds can be biologically opened to form the diazoalkane intermediate. The formation of diazoalkane or other alkylating species is unlikely if the rings cannot be opened (Ref. 5.17). It has been shown that exposure to NPIP, NPYR and NMOR will result in alkylation of RNA in rat liver. These results support the theory that alkylation of nucleic acids is important to the carcinogenic effects of N-nitroso compounds (Ref. 5.17).

In addition to carcinogenic activity, the analytes all have mutagenic effects. In early studies, N-nitrosamines were found to be mutagenic only in tests with Drosophilia melanogaster (Fruit Fly) and no activity was observed in tests using bacteria, yeasts or fungi. The reason for lack of activity in the microorganisms is that Drosophilia can biologically activate nitrosamines while the others cannot. This conclusion is supported by the fact that NDMA is mutagenic to bacteria when incubated with rat liver microsomes. Therefore, in order to exert mutagenic effects, the analytes must be biologically activated (Ref. 5.18).

All seven of the N-nitrosamines covered in this method exert a powerful carcinogenic action on experimental animals. While there is no direct evidence that exposure to the analytes can cause cancer in humans, the wide range of species that is susceptible to their carcinogenic action suggests that man is probably not resistant (Ref. 5.19).

The International Agency for Research on Cancer, (IARC) recommends that all seven of the analytes should be treated as if they were carcinogenic to humans (Ref. 5.14).

1.1.3 Exposure

NDMA has been employed as an industrial solvent and in the synthesis of the rocket fuel 1,1-dimethylhydrazine. Patents and proposed uses include, as an antioxidant, a softener for copolymers, an additive for lubricants, in condensers to increase the dielectric constant and a nematocide (Ref. 5.20). NDEA has been suggested for use as a solvent, a softener for copolymers, a lubricant additive, in electrical condensers and chemical synthesis (Ref. 5.20). NDBA has been tested as a fungicide and used in chemical synthesis (Ref. 5.21).

Patents exist for the use of NMOR as a solvent for polyacrylonitrile and as a intermediate for the synthesis of N-aminomorpholine. NMOR has been found to be an effective agent to combat microbial infections (Ref. 5.14). The use of NDMA was discontinued in 1976 and, at present, there is no evidence that any of the analytes are intentionally produced for other than research purposes (Ref. 5.14).

Some of the analytes have been determined to be present in beers (Ref. 5.22), whiskeys (Ref. 5.23), tobacco (Ref. 5.24), tobacco smoke (Ref. 5.24), herbicides (Ref. 5.25), deionized water (Ref. 5.26), free amines (Ref. 5.27), corrosion inhibitors (Ref. 5.28), diesel engine crankcase emissions (Ref. 5.29) and new car interiors (Ref. 5.30).

Industries in which some of the analytes have been detected include iron foundries (Ref. 5.30), leather tanneries (Ref. 5.31), rubber producing factories (Ref. 5.32) and rubber products manufacturing plants (Ref. 5.32).

N-nitrosamines are extensively used in cancer research facilities. Human exposure can occur when the unchanged agents are excreted by the laboratory animals.

Non-occupational sources of exposure to the analytes are the endogenous formation of the agents in the human gastrointestinal tract. Precursor amines have been shown to react with nitrite to form the corresponding N-nitrosamines under conditions found in the mammalian stomach (Ref. 5.38). NMOR has been produced in vivo by mice gavaged with morpholine and later exposed to nitrogen dioxide in inhalation chambers (Ref. 5.39). The source of volatile N-nitrosamines found in normal human feces, urine and saliva has been suggested to be in vivo nitrosation of ingested secondary and tertiary amines (Refs. 5.40-5.42).

The size of the work population that is exposed is unknown. Since amines and suitable nitrosating species are ubiquitous the number of potential exposures seems large (Ref. 5.43).

Because the analytes are such powerful animal carcinogens, they are probably not used to any great degree by U. S. industry today. Most occupational exposure to the analytes is probably the result of the unintentional formation of the agent from precursor amines and suitable nitrosating species. The amino group can be primary, secondary or tertiary (Ref. 5.36). The amine can be free or a portion of a more complex molecule such as a drug or a herbicide. Amines can be nitrosated in air (Ref. 5.34) or in solution under acidic, neutral or alkaline conditions (Ref. 5.36). The nitrosation reaction is catalyzed by thiocyanate, halide ions, metal ions, formaldehyde and ozone (Refs. 5.35 and 5.36). Suitable nitrosating species include nitrogen oxides (NO, NO_2, N_2O_3, N_2O_4), nitrite and nitrous acid (Ref. 5.36). Nitrosation can occur as a result of transnitrosation. This is a chemical reaction in which a N-nitrosamine transfers its nitroso group to another amine (Ref. 5.37).

1.1.4 Physical properties

The following data were taken from Refs. 5.13 and 5.44.

analyte		NDMA	NDEA	NDPA	NPIP
CAS no.:		62-75-9	55-18-5	621-64-7	100-75-4
molecular weight:		74.08	102.14	130.19	114.15
boiling point, °C/mmHg:		154/760	176.9/760	206/760	217/721
density, g/mL:		1.0059	0.9422	0.9163	1.0631
refractive index:		1.4358	1.4386	1.4437	1.4933
physical appearance:		yellow liquid	yellow liquid	yellow liquid	yellow liquid
solubility	water:	v. sol	v. sol	sol	sol
	alcohol:	v. sol	v. sol	miscible	
	ether:	v. sol	v. sol	miscible	
UV absorption	λ max, nm:	231	233	233	354
	log ε:	3.85	3.7	3.85	1.91
	λ max, nm:	346	350	350	366
	log ε:	2.00	1.95	1.95	2.04
	λ max, nm:				377
	log ε:				2.00

The following data were taken from Refs. 5.13. and 5.14.

analyte		NDBA	NPYR	NMOR
CAS no.:		924-16-3	930-55-2	59-89-2
molecular weight:		158.2	100.2	116.1
boiling point, °C/mmHg:		116/14	214/760	224/747
density, g/mL (20/4):		0.9009		
refractive index:		1.4475		
melting point, °C:				29
physical appearance:		yellow oil	yellow liquid	yellow solid
solubility	water:	0.12%	miscible	miscible
	org. solv.:	sol	sol	sol
	lidips:	sol	sol	
UV absorption	λ max, nm:	233	230	237
	log ε:	2.65	2.91	2.84
	λ max, nm:	347	333	346
	log ε:	0.75	1.03	0.86

Synonyms (Ref. 5.13)

N-nitrosodimethylamine

dimethylnitrosamin (German); dimethylnitrosamine; N,N-dimethylnitrosamine; N-methyl-N-nitrosomethanamine; dimethylnitrosoamine; DMN; DMNA; NDMA.

N-nitrosodiethylamine

diaethylnitrosamin (German); diethylnitrosamine; diethylnitrosoamine; N,N-diethylnitrosamine; ethylamine, N-nitrosodi-; N-ethyl-N-nitroso ethanamine; nitrosodiethylamine; DANA; DEN; DENA; NDEA.

N-nitrosodipropylamine

di-n-propylnitrosamine; N-nitrosodi-n-propylamine; N-nitroso-N-propyl-1-propanamine; propylamine, N-nitroso-N-di-; propanamine, N-nitroso-Npropyl; DPN; DPNA; NDPA.

N-nitrosodibutylamine

butylamine, N-nitrosodi-; N-butyl-N-nitroso-1-butamine; dibutylamine, N-nitroso-; di-n-butylnitrosamin (German); dibutylnitrosamine; di-n butylnitrosamine; N,N-di-n-butylnitrosamine; N-nitroso-di-n-butyl amine; DBN; DBNA; NDBA.

N-nitrosopiperidine

nitrosopiperidin (German); N-nitroso-piperidin (German); 1-nitrospi peridine; NO-Pip; NPIP.

N-nitrosopyrrolidine

N-nitrosopyrrolidin (German); pyrrolidine, 1-nitroso-; NO-Pyr; NPYR.

N-nitrosomorpholine

morpholine, 4-nitroso-; N-nitrosomorpholin (German); 4-nitrosomorpho line; NMOR.

molecular formula (Ref. 5.14)

NDMA - $(CH_3)_2NNO$
NPIP - PIP-NO ($C_5H_{10}N_2O$)
NDEA - $(C_2H_5)_2NNO$
NPYR - PYR-NO ($C_4H_8N_2O$)
NDPA - $(C_3H_7)_2NNO$
NMOR - MOR-NO ($C_4H_8N_2O_2$)
NDBA - $(C_4H_9)_2NNO$

See Figure 1.1.4 for the molecular structures of the analytes.

1.2 Limit defining parameters

The air samplers were vapor spiked with the analytes by the liquid injection of a certified mixture on Polar Partition tubes. The spiked tubes were placed in front of ThermoSorb/N air samplers and then 50 L (1 L/min) of air, at about 80% relative humidity and 22°C, were drawn through the sampling train. The N-nitrosamines were vaporized from the Polar Partition tubes and collected on the ThermoSorb/N tubes. The vaporization process was shown to be complete after about 25 L had passed through the Polar Partition tubes.

The analytes were vapor spiked on the air samplers as components of a commercially prepared, certified mixture. The mixture was prepared and certified by Thermo Electron Corporation and their results are presented below.

Concentrations of the Certified Mixture in µg/mL

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
10	10	10	9	9	10	15

1.2.1 Detection limits of the analytical procedure

The detection limit of the analytical procedure is the amount of analyte per injection which will result in a peak whose height is about 5 times the amplitude of the base line noise.

The detection limits are presented below as mass per injection. (Section 4.1)

The Detection Limits of the Analytical Procedure, pg

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
50	50	50	45	45	50	75

1.2.2 Detection limits of the overall procedure

The detection limit of the overall procedure is the amount of analyte spiked on the sampling device which allows recovery of an amount of analyte equivalent to the detection limit of the analytical procedure. (Section 4.2)

The Detection Limits of the Oveall Procedure

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
ng/sample	10	10	10	9	9	10	15
ppb	0.043	0.031	0.024	0.019	0.026	0.032	0.042
µg/m^3	0.13	0.13	0.13	0.12	0.12	0.13	0.20

All values based on the recommended air volume.

1.2.3 Reliable quantitation limits

The reliable quantitation limit is the smallest amount of analyte which can be quantitated within the requirements of 75% recovery and 95% confidence limits of ±25%.

The reliable quantitation limits were the same as the detection limits of the overall procedure since the desorption efficiencies were above 75% and the 95% confidence intervals were less than ±25%. (Section 4.2)

The Reliable Quantitation Limits

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
ng/sample	10	10	10	9	9	10	15
ppb	0.043	0.031	0.024	0.019	0.026	0.032	0.042
µg/m^3	0.13	0.13	0.13	0.12	0.12	0.13	0.20

All values based on the recommended air volume.

The reliable quantitation limit and detection limits reported in the method are based upon optimization of the instrument for the smallest possible amount of analyte. When the target concentration of an analyte is exceptionally higher than these limits, they may not be attainable at the routine operating parameters.

1.2.4 Sensitivity

The sensitivity of the analytical procedures is determined by the slope of the calibration curves over a concentration range from 0.5 to 2 times the target concentrations. The sensitivity will vary somewhat with the particular instrument used in the analysis. (Section 4.4)

The Sensitivity of the Analytical Procedures

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
area count per µg/mL	239864	208144	163134	129648	179720	201661	176596

1.2.5 Recovery

The recovery of the analytes from the collection medium must be 75% or greater. The average recoveries from spiked samples over the range of 0.5 to 2 times the target concentrations are presented below. (Section 4.6)

Average Desorption Efficiencies, %

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
97.4	99.7	98.9	97.7	96.0	97.6	98.1

1.2.6 Precision (analytical method only)

The pooled coefficients of variation obtained from replicate determinations of analytical standards at 0.5, 1 and 2 times the target concentrations are presented below. (Section 4.3)

The Pooled Coefficients of Variation

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0.037	0.044	0.048	0.047	0.051	0.040	0.032

1.3 Advantages

1.3.1 The sampling and analytical procedures permit the simultaneous determination of a mixture of analytes.

1.3.2 The sampling and analytical procedures are precise, reliable, safe and convenient.

1.3.3 The sampling procedure is artifact free. The capacity of the sampling device to prevent artifacts is probably not unlimited.

1.3.4 The air sampling device is commercially available and is constructed of opaque plastic to prevent photo-degradation of the collected N-nitrosamines.

1.3.5 The samples are stable when stored at ambient temperatures for at least 17 days.

1.4 Disadvantages

1.4.1 Smaller laboratories may not be able to support the cost of the recommended sampling and analytical instrumentation.

1.4.2 The ability of the sampling device to collect and retain the analytes is limited.

2. Sampling Procedure

2.1 Apparatus

2.1.1 An air sampling pump, the flow of which can be determined to within ±5% at the recommended air flow rate with the air sampler in line.

2.1.2 ThermoSorb/N air sampling cartridges available from Thermo Electron Corporation, Waltham, Mass.

2.1.3 Equipment to measure the air flow rate through the sampling device.

2.2 Reagents

None required

2.3 Technique

2.3.1 Prior to sampling, remove the ThermoSorb/N air sampling device from the foil container. Save the container so it can be used for sample shipment.

2.3.2 Prior to sampling, remove the red end caps from the inlet and outlet ports. Store the caps on the air sampler in the places that are provided for this use.

2.3.3 Label the air sampler and attach the device to the air sampling pump with flexible tubing. Adjust the pump to obtain the proper air flow rate. The recommended rate is 1 L/min, but flow rates of from 0.2 to 2 L/min may be used. If air volumes larger than the recommended 75 L are to be sampled or if large amounts of N-nitrosamines are suspected, two air samplers may be connected in series. Always calibrate the pump with the sampling device in line.

2.3.4 Attach the sampling device in the breathing zone of the employee to be monitored. The molded clip is convenient for this purpose.

2.3.5 After sampling for the appropriate time, remove the device and replace the red end caps on the inlet and outlet ports of the sampler.

2.3.6 Wrap each sample end to end with official OSHA seals. Place the sealed air sampler inside the foil container from Section 2.3.1.

2.3.7 With each set of samples, submit at least one blank sample. The blank should be subjected to the same handling as the sample except that no air is drawn through it.

2.3.8 Place the samples in a freezer if they are to be stored before shipping to the laboratory.

2.3.9 List possible interferences on the sample data sheet.

2.4 Breakthrough

Breakthrough studies were conducted by connecting two air samplers in series. The first air sampler was vapor spiked with twice the target concentration of the mixture and then air at 80% relative humidity and 22°C was drawn through the sampling train. Both air samplers were analyzed after the appropriate air volume had been sampled. Percent breakthrough was defined as the amount of a component found on the second ThermoSorb/N tube divided by the amount of that component vapor spiked on the first tube, multiplied by 100.

NDMA was the only component of the mixture that was lost from the first sampling device. Five-percent breakthrough occurred after about 100 L of air had been sampled. The experiment was repeated with NDMA as the only analyte vapor spiked and similar results were obtained. These results indicate the capacity of the tube was not exceeded by the mixture (3 µg of N-nitrosamines). The manufacturer reports that 1500 µg of N-nitrosamines may exceed the capacity of a single tube (Ref. 5.45).

It was found that as much as 280 L of air may be sampled for NDMA with no loss of the agent if two ThermoSorb/N tubes were connected in series. When 400 L of air were sampled at 2 L/min, about 16% of the NDMA vapor spiked on the first tube was lost from the sampling train. All of the recovered NDMA was found on the second tube. None of the other components moved from the first tube.

When the relative humidity was decreased from 80%, the 5% breakthrough air volume for NDMA was found to increase. At 63% relative humidity, over 200 L of air was sampled with no NDMA breakthrough. The complete results of the breakthrough study are presented in Section 4.5.

2.5 Desorption efficiency

The average desorption efficiency for each of the analytes vapor spiked at 0.5, 1 and 2 times the target concentration on ThermoSorb/N air samplers is presented below. (Section 4.6)

Average Desorption Efficiencies, %

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
97.4	99.7	98.9	97.7	96.0	97.6	98.1

2.6 Recommended air volume and sampling rate

2.6.1 The recommended air volume is 75 L.

2.6.2 The recommended air sampling rate is 1 L/min. Studies indicate that sampling rates of from 0.2 to 2 L/min may be used.

2.7 Interferences (sampling)

2.7.1 It is imperative, when sampling air for nitrosamines, to be certain that the analyte is indeed present in the air and not artifactually formed on the sampling device from precursor amines and nitrosating agents. The commercial ThermoSorb/N N-nitrosamine air sampler is constructed to prevent the in situ formation of N-nitrosamines from airborne precursors. The manufacturer has tested the sampling device for the artifactual formation of NDMA, NPYR, NMOR and diisopropylnitrosamine using 50 µg each of the respective precursor secondary amines and nitrogen oxides (NO_x). The amines were spiked on the ThermoSorb/N tubes and then 100 L of air containing 0.2, 0.5, 1, 2 or 4 ppm NO/NO_2 at a 1/1 ratio was sampled. Various untreated solid adsorbents formed the N-nitrosamines quite easily but at these levels of amines and NO_x, the ThermoSorb/N cartridges were shown to be artifact-free (Ref. 5.9).

The process by which the ThermoSorb/N tubes prevent artifact formation is proprietary. The manufacturer states that the device has an amine trap that removes incoming amines and holds them unavailable for nitrosation. The sampled air, after passing through the amine trap, enters a solid sorbent bed where the N-nitrosamines are quantitatively removed and retained. The sampled air next passes through fiber pads containing a nitrosation inhibitor and finally exits the sampler. When the air sampling device is backflushed with solvent, the nitrosation inhibitor is put into solution and is washed together with the collected N-nitrosamines and amines into a collection vial. The nitrosation inhibitor successfully competes with the amine and chemically removes any nitrosating species (Ref. 5.46).

The artifact resistance of the ThermoSorb/N air sampler was verified using morpholine (a precursor amine for NMOR) and nitrogen dioxide. Morpholine was selected as the test amine because it has been shown to be easily nitrosated. Nitrogen dioxide in a gas bag, mixed with humid air, has been shown to be an effective nitrosating species. A Teflon gas bag was prepared containing 33 µg/L morpholine in nitrogen and another containing 4.8 ppm (v/v) NO_2 in air at about 75% relative humidity was also prepared. The morpholine bag was sampled for 50 min at 1 L/min and then the NO_2 bag was sampled for 50 min at 1 L/min. The total morpholine loaded on the tube was 1650 µg. There was no NMOR formed on the ThermoSorb/N cartridge. The experiment was repeated using a standard Florisil tube. The result showed that 7 µg of NMOR could be formed, as an artifact on the tube, after sampling each bag for 7 L.

2.7.2 It is unknown if there are other potential interferences with the collection of N-nitrosamines using ThermoSorb/N cartridges.

2.8 Safety precautions (sampling)

2.8.1 Attach the sampling equipment to the worker in such a manner that it will not interfere with work performance or safety.

2.8.2 Follow all safety practices that apply to the work area to be monitored.

3. Analytical Procedure

3.1 Apparatus

3.1.1 A temperature programmable gas chromatograph (GC).

3.1.2 A high performance liquid chromatographic (HPLC) pump.

3.1.3 An HPLC sample injector.

3.1.4 A Thermal Energy Analyzer (TEA), Thermal Electron Corporation, Waltham, Mass.

3.1.5 A GC column capable of resolving the analytes from each other and potential interferences. The column used in this work was a 10-ft × 1/8-in. stainless steel column containing 10% Carbowax 20M with 2% KOH on 80/100 mesh Chromosorb W AW.

3.1.6 A HPLC analytical column capable of resolving the analytes from each other and potential interferences. The column used in this work was a DuPont Zorbax CN, 4.6 mm × 25 cm.

3.1.7 The necessary hardware to interface the TEA to the GC and the HPLC apparatus.

3.1.8 An electronic integrator or other suitable means to measure peak area and record chromatograms.

3.1.9 Vials, 2-mL with Teflon-lined caps.

3.1.10 Syringes, of convenient sizes for samples and standard preparations and injections.

3.1.11 Hypodermic needles, 23 gauge × 1 in. with a Luer hub.

3.1.12 Volumetric flasks, 1-mL and other convenient sizes.

3.1.13 Dewar flasks, of convenient sizes for liquid nitrogen.

3.1.14 Heating tape, high temperature, heavily insulated.

3.1.15. Variable voltage transformer.

3.2 Reagents

3.2.1 NDMA, NDEA, NDPA, NDBA, NPIP, NPYR and NMOR analytical standards. A certified mixture containing each of the analytes, in ethanol, at the following concentrations was used to evaluate this method. The mixture was purchased from and certified by Thermo Electron Corporation, Waltham, Mass.

Concentrations of the Certified Mixture in µg/mL

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
10	10	10	9	9	10	15

3.2.2 Dichloromethane and methanol, HPLC grade. The sample desorption solution is composed of 75% dichloromethane and 25% methanol by volume.

3.2.3 n-Propanol, acetone and trimethylpentane, HPLC grade.

3.2.4 Nitrogen, liquid.

3.2.5 Helium, GC grade.

3.2.6 Oxygen and air, medical grade.

3.3 Standard preparation

3.3.1 Keep the exposure of the standards to light at a minimum because light will decompose each of the analytes.

3.3.2 In the event that the mixture is not available, dilute the pure individual components to result in a solution at approximately the following concentrations. Dilute concentrations higher than 15 µg/mL with ethanol. When the concentration falls below 15 µg/mL, dilute with the desorbing solution described in Section 3.2.2.

Target Concentrations, µg/mL

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0.20	0.20	0.20	0.18	0.18	0.20	0.30

The target concentration solution was prepared by diluting the standard mixture described in Section 3.2.1 1 to 50 with desorbing solution.

3.3.3 Additional standards at other than the target concentration should be prepared in order to generate the calibration curve.

3.3.4 Store the standards using well sealed, dark containers in a freezer.

3.4 Sample preparation

3.4.1 Store the samples in a freezer until analysis.

3.4.2 The sample should be received in a foil container. Remove the sample from the container.

3.4.3 Insure that the official OSHA seal is intact and complete.

3.4.4 Check the laboratory sample number against the field identification number to be sure that the sample has been properly identified.

3.4.5 Prepare the desorption solution described in Section 3.2.2. Store the solution in a well sealed, dark bottle.

3.4.6 Label two 1-mL volumetric flasks with the sample number. Further label one flask "A" and the other "B".

3.4.7 Remove the OSHA seal and the red end caps from the sample.

3.4.8 Attach a syringe needle to the male Luer fitting at the inlet port of the air sampler.

3.4.9 Fill a syringe with about 4 mL of the desorption solution. Attach the syringe to the female Luer fitting located at the outlet end of the air sampler.

3.4.10 Elute the sample by gently forcing the desorption solvent through the air sampler at approximately 0.5 mL/min. Collect the first 1-mL portion of solvent in the volumetric flask labeled "A" in Section 3.4.6 and the second 1-mL of eluent in the flask labeled "B".

3.4.11 Because light will decompose N-nitrosamines, it is necessary to prevent exposure of the eluted samples to light.

3.4.12 If the eluted samples are not to be analyzed immediately, transfer the contents of each flask to a separate vial which can be sealed with a Teflon-lined cap. Protect the samples from light and store them in a freezer.

3.5 Analysis

3.5.1 Instrument conditions

GC conditions

injector temperature: 150°C
column temperature: 150 to 200°C at 4 °C/min
transfer line temp.: 210°C
helium (carrier gas): 30 mL/min
injection volume: 5 µL

The recommended GC column is a 10-ft × 1/8-in. stainless steel column packed with 10% Carbowax 20M with 2% KOH on 80/100 mesh Chromosorb W AW.

TEA conditions

GC pyrolyzer temp.:	475°C
oxygen:	5 mL/min
pressure:	1 mm Hg
attenuation:	4
cold trap temp.:	-130°C (n-propanol and liquid nitrogen)

3.5.2 The transfer line between the GC and the TEA must be maintained at about 210°C. The use of a heating tape and a variable voltage transformer is recommended.

3.5.3 Chromatogram (Figure 4.3.1)

3.5.4 Detector response is measured with an electronic integrator or other suitable means.

3.5.5 An external standard procedure is used to prepare a calibration curve using at least three standard solutions of different concentrations. The calibration curve is pre pared daily. The integrator is calibrated to report results in µg/mL.

3.5.6 Bracket the samples with analytical standards.

3.6 Interferences (analytical)

3.6.1 Because the TEA has been shown to respond to compounds other than N-nitrosamines (Ref. 5.47), it is strongly recommended that positive GC/TEA results be confirmed by HPLC/TEA analysis. Since GC and HPLC separation techniques operate using different principles, the component elution order is not the same. It is unlikely that two different compounds will have a coincidence of retention time on both GC and HPLC columns.

HPLC conditions for confirmation of high samples

column:	DuPont Zorbax CN (4.6 mm × 25 cm)
mobile phase:	95% trimethylpentane/5% acetone, v/v
flow rate:	1.3 mL/min
injection volume:	5 to 25 µL

The TEA conditions are the same as for the GC/TEA analysis except that the HPLC pyrolyzer is used at 550°C and the cold trap is maintained at -80°C with a mixture of water, n-propanol and liquid nitrogen.

chromatogram: Figure 4.3.2

3.6.2 GC and HPLC parameters may be changed to circumvent interferences. Possible interferences are listed on the sample data sheets.

3.6.3 The only unequivocal means of structure designation is by high resolution mass spectrometry with continuous peak matching. It is recommended this procedure be used to confirm samples whenever possible.

3.7 Calculations

3.7.1 The integrator value in µg/mL is used for reference only. More reliable results are obtained by use of a calibration curve. The detector response, for each standard, is compared to its equivalent concentration in µg/mL and the best straight line through the data points is determined by linear regression.

3.7.2 The concentration, in µg/mL, for a particular determination is obtained by comparing its detector response to the calibration curve.

3.7.3 The result obtained from the analysis of each vial or flask is corrected by the appropriate desorption efficiency, and then the corrected results from the "A" and "B" determinations that compose a particular air sample are added together.

3.7.4 The air concentrations for a sample result is calculated by the following equation:

$\mu g/m^3 = (C)(D)(1000)/E$

where C = µg/mL from Section 3.7.3
D = desorption volume in milliliters (1 mL)
E = air volume in liters

3.7.5 To correct the results from Section 3.7.4 to parts-per-billion the following relationship is used:

$ppb = (\mu g/m^3)(24.46)/MW$

where $\mu g/m^3$ = result from Section 3.7.4
24.46 = molar volume of an ideal gas at 25°C and 760 mm Hg.
MW = molecular weight of the analyte, obtained below.

Molecular weights of the Analytes

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
74.08	102.14	130.19	158.2	114.15	100.2	116.1

3.8 Safety precautions (analytical)

3.8.1 The analytes are extremely potent animal carcinogens and utmost care must be exercised when working with these compounds.

3.8.2 Avoid skin contact with liquid nitrogen and the solvents.

3.8.3 Confine the use of solvents to a fume hood.

3.8.4 Wear safety glasses in all laboratory areas.

3.8.5 Check to be sure that the TEA exhaust is connected to a fume hood.

4. Backup Data

4.1 Detection limit of the analytical procedure

Figure 4.1 is a chromatogram obtained from a 5-µL injection of a standard solution at the following concentrations.

Table 4.1
The Concentrations of the Detection Limit Standard
in µg/mL

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0.01	0.01	0.01	0.009	0.009	0.01	0.015

4.2 The detection limit of the overall procedure and the reliable quantitation limit.

The following data were obtained by vapor spiking the analytes on air samplers.

Table 4.2
Desorption Efficiency at the Detection Limit

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
ng/sample	10	10	10	9	9	10	15
recovery,%	91.0	107.2	90.1	113.2	95.4	83.5	103.1
	101.2	116.5	90.4	96.0	94.7	81.0	98.1
	102.7	99.5	93.3	98.3	95.0	79.1	85.5
	104.4	112.7	101.3	119.5	103.2	98.1	101.0
	109.5	108.4	103.8	100.8	102.2	94.6	90.9
$\bar{X}$	108.1	112.0	96.2	104.7	92.4	96.7	86.0
SD	102.8	109.4	95.8	105.4	97.2	88.8	94.1
1.96 SD	6.593	5.864	5.697	9.166	4.435	8.550	7.674
	12.9	11.5	11.2	18.0	8.7	16.8	15.0

4.3 Precision of the analytical procedure

The following data were obtained from multiple injections of analytical standards.

Table 4.3.1
0.5× Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/mL	0.10	0.10	0.10	0.090	0.090	0.10	0.15
area	25396	20623	16848	11244	16015	20051	26683
counts	26599	22521	18318	11693	16543	19715	26215
	24888	23689	17322	11788	14112	18453	28041
	24455	21599	17349	12042	15843	19973	27430
	27643	20024	16197	12855	15714	17380	26656
$\bar{X}$	25796.2	21691.2	17206.8	11924.4	15645.4	19114.2	27005.0
SD	1307.57	1467.04	777.28	594.76	913.42	1163.94	725.17
CV (%)	5.07	6.76	4.52	4.99	5.84	6.09	2.69

Table 4.3.2
1× Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/mL	0.20	0.20	0.20	0.18	0.18	0.20	0.30
area	49510	43828	28483	20855	29517	37646	56148
counts	50742	42661	32234	24048	33733	38898	52347
	47671	40916	29839	23085	32097	38797	53250
	47182	40588	28192	21746	32614	39735	55774
	51124	43604	32308	23588	34985	39681	56741
$\bar{X}$	49245.8	42319.4	30211.2	22664.4	32589.2	38951.4	54852.0
SD	1773.08	1500.84	1980.57	1328.69	2045.08	848.10	1932.58
CV (%)	3.60	3.55	6.56	5.86	6.28	2.18	3.52

Table 4.3.3
2× Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/mL	0.40	0.40	0.40	0.36	0.36	0.40	0.60
area	97764	84833	63298	45551	61866	80671	104244
counts	97759	82918	64629	46465	64343	77716	109657
	96497	83760	65206	46143	64646	81891	108013
	100393	84837	67017	46978	64918	77044	101980
	95827	84281	67276	48762	65845	80412	109832
$\bar{X}$	97648.0	84125.8	65485.2	46779.8	64323.6	79546.8	106745.2
SD	1746.54	809.72	1669.45	1223.00	1484.26	2069.03	3484.87
CV (%)	1.79	0.96	2.55	2.61	2.31	2.60	3.26

Table 4.3.4
The Pooled Coefficients of Variation

NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0.037	0.044	0.048	0.047	0.051	0.040	0.032

4.4 Sensitivity

The data in Tables 4.3.1 - 4.3.3 are presented respectively in Figures 4.4.1 - 4.4.7.

4.5 Breakthrough

The breakthrough study was conducted by first vapor-spiking the following amounts of the analytes on the first of two ThermoSorb/N tubes connected in series.

Table 4.5.1
Amounts of Analyte Used in Breakthrough Studies

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg	0.40	0.40	0.40	0.36	0.36	0.40	0.60

Next, air at about 80% relative humidity and 22°C was drawn through the sampling train. After the appropriate air volume had been sampled, both tubes were analyzed and the results are presented in Table 4.5.2.

Table 4.5.2
Breakthrough Study

air vol	flow rate	% breakthrough						
(L)	(L/min)	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
80	1.0	0	0	0	0	0	0	0
88	0.2	4.3	0	0	0	0	0	0
93	2.0	0	0	0	0	0	0	0
120	1.0	16.0	0	0	0	0	0	0
128[a]	1.0	19.2						
280[b]	1.0	93.7	0	0	0	0	0	0
400[c]	2.0	100.0	0	0	0	0	0	0

[a] This run (with NDMA only) was made to determine if the presence of the other analytes had an effect on the breakthrough for NDMA.
[b] NDMA lost from the first tube was recovered in the second tube.
[c] About 16% of the NDMA vapor-spiked was lost from the system.

4.6 Desorption efficiency (vapor-spiked)

Table 4.6.1
Desorption Efficiency at 0.5× the Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/sample	0.10	0.10	0.10	0.09	0.09	0.10	0.15
%	10.53	99.10	99.70	95.24	92.33	93.19	92.33
recovered	92.10	95.50	96.40	93.73	95.15	93.19	94.07
	93.20	98.08	95.95	96.24	93.20	91.43	94.36
	92.65	101.77	98.04	92.65	92.86	94.09	101.81
	100.67	95.58	96.83	101.03	100.00	96.05	96.92
	94.88	100.48	97.91	100.00	94.57	101.97	100.15
$\bar{X}$	95.67	98.42	97.47	96.48	94.68	94.99	96.61

Table 4.6.2
Desorption Efficiency at 1× the Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/sample	0.20	0.20	0.20	0.18	0.18	0.20	0.30
%	106.84	107.49	101.00	102.03	98.45	99.28	100.87
recovered	98.19	99.16	102.68	101.82	101.46	100.00	101.87
	96.60	102.40	103.43	101.60	98.45	96.73	97.48
	92.10	107.00	96.00	96.89	96.67	93.00	100.18
	103.59	96.45	104.50	103.33	93.06	97.92	97.67
	96.50	94.32	95.91	99.86	95.37	99.11	94.16
$\bar{X}$	98.97	101.14	100.59	100.92	97.24	97.67	98.56

Table 4.6.3
Desorption Efficiency at 2× the Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg/sample	0.40	0.40	0.40	0.36	0.36	0.40	0.60
%	95.78	100.51	98.46	95.20	96.58	98.77	96.14
recovered	99.22	101.33	100.00	97.11	99.14	100.56	99.72
	97.21	95.37	98.41	98.50	96.01	97.88	100.80
	94.15	99.02	99.39	94.66	94.15	100.99	95.76
	99.22	100.62	97.54	94.22	95.76	101.36	100.37
	98.99	100.32	98.16	94.36	94.15	101.36	101.34
$\bar{X}$	97.43	99.53	98.66	95.68	95.96	100.15	99.02

Table 4.6.4
Average Desorption Efficiency Over the
Range of from 0.5× to 2× the Target Concentration

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
%	97.4	99.7	98.9	97.7	96.0	97.6	98.1

4.7 Storage test

The data in Tables 4.7.2 and 4.7.3 represent the effects of storage at ambient (21 to 26°C) and reduced (-20°C) temperatures on the analytes vapor spiked on ThermoSorb/N tubes at the following levels.

Table 4.7.1
Amounts of Analyte Used in Storage Tests

analyte	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
µg	0.20	0.20	0.20	0.18	0.18	0.20	0.30

The recoveries are not corrected for desorption efficiency. Three stored samples were analyzed at approximately three-day intervals. Graphical representations of the data may be found in Figures 4.7.1 to 4.7.14.

Table 4.7.2
Ambient Temperature Storage Test (% Recovered)

days of storage	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0	94.0	97.0	97.2	96.2	94.8	94.8	92.4
	99.7	104.2	104.0	99.4	101.9	100.4	99.3
	100.2	102.0	102.1	98.6	99.0	95.3	95.0
3	95.8	98.9	94.3	96.4	94.2	91.5	89.5
	97.2	92.7	97.4	91.0	93.0	88.2	89.6
	99.0	95.8	92.6	93.7	95.2	91.9	97.9
7	98.5	96.6	101.0	92.1	94.6	90.6	90.3
	102.4	100.9	97.2	10.7	93.6	89.7	92.1
	93.9	100.5	98.0	99.1	96.6	93.0	99.9
10	95.7	96.2	99.0	92.4	93.6	85.9	96.2
	97.0	96.8	92.6	89.1	90.0	85.4	90.8
	103.2	108.2	104.0	97.7	99.3	94.8	97.7
14	93.5	95.0	89.0	83.5	89.0	86.0	88.0
	96.3	99.3	93.8	88.9	89.2	86.3	88.8
	94.1	96.3	93.1	87.8	86.0	84.3	85.1
17	92.3	97.6	92.2	91.4	89.4	86.0	90.2
	91.5	96.9	92.8	88.9	90.2	85.6	89.7
	93.2	100.1	92.4	89.7	89.3	84.9	89.8

Table 4.7.3
Reduced Temperature Storage Test (% Recovered)

days of storage	NDMA	NDEA	NDPA	NDBA	NPIP	NPYR	NMOR
0	96.4	100.4	99.1	100.6	97.8	91.5	94.5
	95.8	99.4	94.6	95.1	95.0	90.9	95.2
	94.3	96.6	93.1	97.0	94.2	89.5	90.6
3	95.2	106.0	105.7	101.2	100.4	97.9	98.8
	96.5	98.5	98.2	95.1	94.3	95.0	91.2
	97.7	101.1	100.2	97.6	95.4	93.0	93.5
7	101.4	100.3	100.0	92.6	97.1	96.7	96.0
	99.9	99.4	97.5	93.3	96.3	92.6	90.2
	99.5	100.5	97.8	94.1	97.3	92.3	94.9
11	103.0	105.3	101.2	94.7	101.9	91.4	94.7
	99.5	103.2	101.9	102.6	99.1	94.8	94.3
	99.7	100.1	97.5	96.2	94.5	89.0	90.2
14	101.6	103.5	104.8	107.5	101.0	96.7	94.8
	105.1	101.6	100.8	99.2	96.3	86.8	89.5
	100.3	103.1	100.7	100.1	100.8	94.1	93.0
17	99.6	101.4	97.6	99.7	101.6	94.7	94.1
	95.4	97.1	92.2	96.4	96.4	92.5	92.5
	97.3	100.5	100.1	96.1	95.9	89.3	98.3

4.8 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy).

NDMA
The LD_{50} for NDMA, administered orally to the rat, is 26 mg/kg. The LC_{50} is 78 ppm following an exposure time of four hours (Ref. 5.13). NDMA is carcinogenic to many different animal species. The agent is carcinogenic following prenatal exposure and in single dose experiments (Ref. 5.14). When 4 mg/kg of NDMA was administered in the drinking water 5 times a week to 20 rats, liver cancer was detected in 13 of the animals. The mean total dose which was applied until the appearance of malignant tumors in 50% of the animals (D_{50}) was 0.4 g/kg and the mean tumor induction period in days (t_{50}) was 270 days. In an inhalation experiment, six rats were exposed to 4 mg/kg NDMA for 1/2 hour two times a week. Four rats died because of cancer of the posterior nasal cavity which had entered the cranial cavity. One rat had cancer of the pituitary gland and the

last animal had a kidney tumor. There were no liver tumors. The D_{50} was 0.25 g/kg and the t_{50} was 400 days. The inhalation experiment was repeated at 2 mg/kg using 12 rats. Three rats died prematurely and a fourth without tumors. The remaining 8 animals died from cancer of the posterior nasal cavity. Again, there were no liver tumors. The D_{50} was lower than for the high dose at 0.14 g/kg and the t_{50} was shorter at 340 days (Ref. 5.12). NDMA has been shown to cause cancer in the rat, mouse, Syrian Golden and European hamsters, mastomys, guinea pig, rainbow trout, newt, aquarium fish and mink. The principal affected organs were the liver, lung, nasal cavities and kidney (Ref. 5.15). It is known that chronic exposure to NDMA causes severe liver damage and cirrhosis in man (Ref. 5.15). It has been reported that both rat and human liver slices metabolize NDMA in a similar manner (Ref. 5.14).

NDEA

The LD_{50} for NDEA is 280 mg/kg after oral administration to the rat (Ref. 5.13). The agent is carcinogenic to many different animal species which include sub-human primates. NDEA induces cancer following prenatal exposure and in single dose experiments (Ref. 5.14). NDEA was administered to rats in drinking water in a chronic exposure experiment. Daily exposure was between 0.075 and 14.2 mg/kg in nine groups of animals. The total dose, until death occurred, was between 64 and 965 mg/kg. The tumor induction time was between 68 and 840 days. All daily doses higher than 0.15 mg/kg gave a tumor incidence of 100%. When a dose of 0.15 mg/kg per day was administered, a tumor yield of 90% was obtained. At 0.075 mg/kg per day, 20 rats survived for more than 600 days and 11 of the 20 animals had tumors of the liver, esophagus, or the nasal cavity. All four of the animals that lived longer than 940 days at this dose level had tumors. The location of the tumors was dependent on the dose given, the non-liver tumors evolved only if cancer of the liver had not caused death. NDEA has proven to be a stronger carcinogen than NDMA even though the LD_{50} for NDMA is much lower (Ref. 5.12). NDEA has been shown to cause cancer in the rat, African white-tailed rat, mouse, Syrian Golden, Chinese and European hamsters, guinea pig, rabbit, parakeet and monkey. The principal affected organs were the liver, esophagus, nasal cavities, kidney, forestomach, lung and larynx (Ref. 5.15).

NDPA

The LD_{50} for NDPA, administered orally to the rat, is 480 mg/kg (Ref. 5.13). In a chronic experiment, the agent was added to the daily food of the rats. Four dose groups were involved - 30 mg/kg led to cancer of the liver, 15 mg/kg produced liver cancer in all the dosed rats and in four cases metastases into the lung was observed. When 8 mg/kg NDPA was administered, the result was cancer in 15 of 16 animals. The lowest dose, 4 mg/kg, resulted in cancer of the liver in 12 of 14 animals. The mean carcinogenic doses (D_{50}) were 3.2, 1.86, 1.52 and 1.15 g/kg. The mean induction periods (t_{50}) were 120, 155, 202 and 300 days. (Ref. 5.12).

NDBA

The LD_{50} for NDBA, following oral administration to the rat, is 1200 mg/kg (Ref. 5.13). When NDBA was given in the food - 75 mg/kg caused liver tumors together with cirrhosis in all the rats, 37.5 mg/kg resulted in liver cancer without cirrhosis in 13 of 16 rats. Five animals had cancer of the esophagus and five cancer of the urinary bladder. Another group of rats received daily doses of 20 mg/kg and the results were multiple cancers of the esophagus in eight rats, seven bladder cancers and four animals with liver cancer. A daily dose of 10 mg/kg produced liver cancer in one animal and two adenomas in a total of ten treated animals. NDBA was the only N-nitrosamine of the 65 tested which produced tumors of the urinary bladder and the agent was particularly effective following subcutaneous injection (Ref. 5.12). NDBA has been shown to cause cancer in the rat, mouse, Syrian Golden and Chinese hamsters and guinea pig. The principal organs affected were the bladder, esophagus, liver forestomach, tongue and lung (Ref. 5.15).

NPIP

The LD_{50} for NPIP, after oral administration to the rat, is 200 mg/kg (Ref. 5.13). When 20 mg/kg was given to rats in the daily drinking water, 17 of 20 rats died prematurely. Three rats died after 186, 232 and 289 days of liver cancer with metastases in the lungs, the last animal also had cancer in the esophagus. Because the 20 mg/kg daily dose was not tolerated well, the experiment was repeated at 5 mg/kg. Of nine animals, one died with papillomas, the other eight died of esophageal cancer. Three rats also had liver cancer. The mean carcinogen dose was 1.4 g/kg. and the average induction period was 280 days (Ref. 5.12). NPIP has produced cancer in the rat, mouse, Syrian Golden hamster and monkey. The principal affected organs were the esophagus, liver, nasal cavities, larynx, trachea and forestomach (Ref. 5.15).

NPYR

The LD_{50} for NPYR, administered orally to the rat, is 900 mg/kg (Ref. 5.13). In chronic experiments, 10 mg/kg and 5 mg/kg were administered in the daily drinking water to two groups of five and 20 rats. Because the carcinogenic effect was weak, the dose was doubled after 150 days. All the animals, but two who died without tumors, developed liver cancer. The average carcinogenic doses (D_{50}) were 4.2 and 3.9 g/kg and the mean induction periods (t_{50}) were 290 and 470 days. The agent was determined to be a weak but relatively certain carcinogen (Ref. 5.12). NPYR has produced cancer in the rat, mouse and Syrian Golden hamster. The principal affected areas were the liver, nasal cavities, testes, lung and trachea (Ref. 5.15).

NMOR

The LD_{50} for NMOR, after oral administration to the rat, was 320 mg/kg (Ref. 5.13). When 16 rats were given 8 mg/kg NMOR daily in the drinking water, 14 animals died of liver cancer. The mean induction period (t_{50}) was only 165 days. A dose of 16 mg/kg caused liver tumors in two of four rats after only 45 and 65 days respectively and after 115 days liver cancer was observed. NMOR was clearly the most rapidly acting liver carcinogen of the 65 N-nitroso compounds tested (Ref. 5.12). NMOR has produced cancer in the rat, mouse, and Syrian Golden hamster. The principal affected organs were the liver, nasal cavities, kidney, esophagus, ovary, trachea, larynx and bronchi (Ref. 5.15).

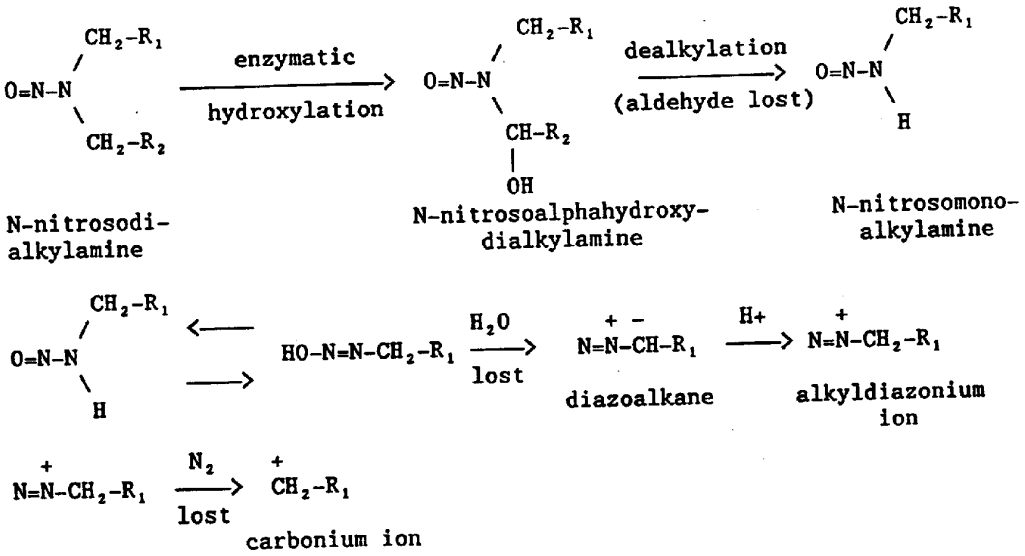


Figure 1.1.2. The metalbolic activation of N-nitrosodialkylamines.

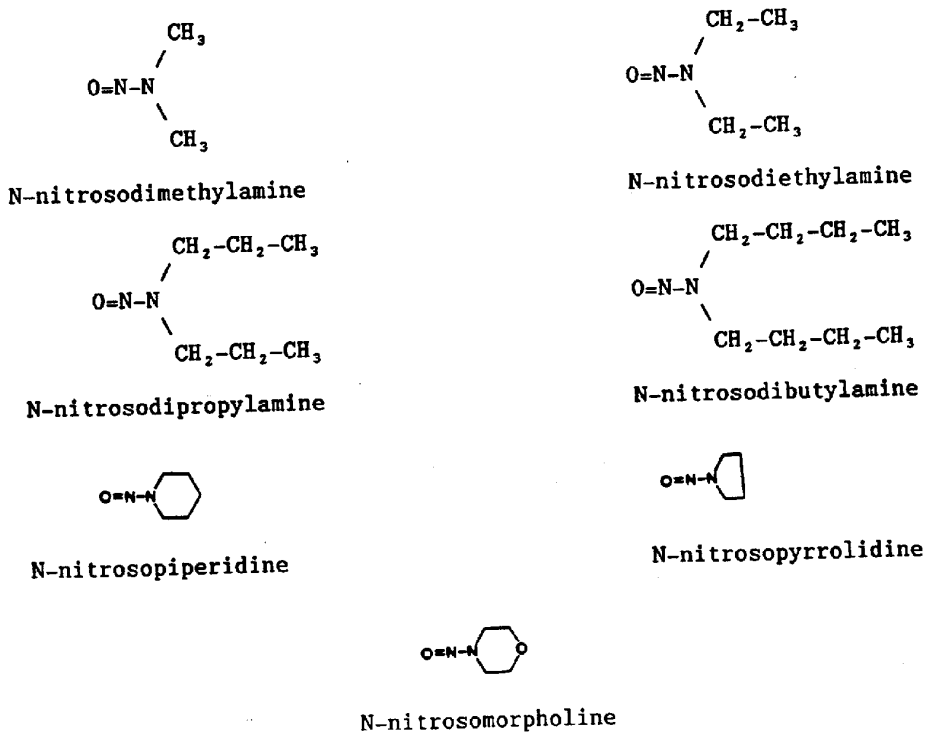


Figure 1.1.4. The molecular structures of the analytes.

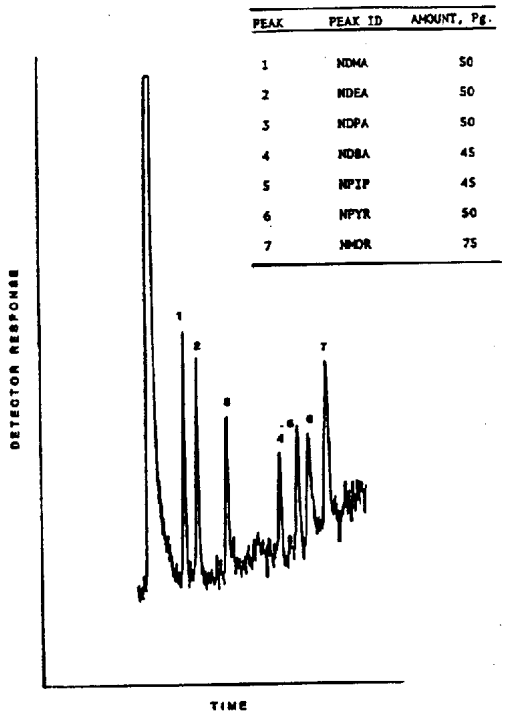
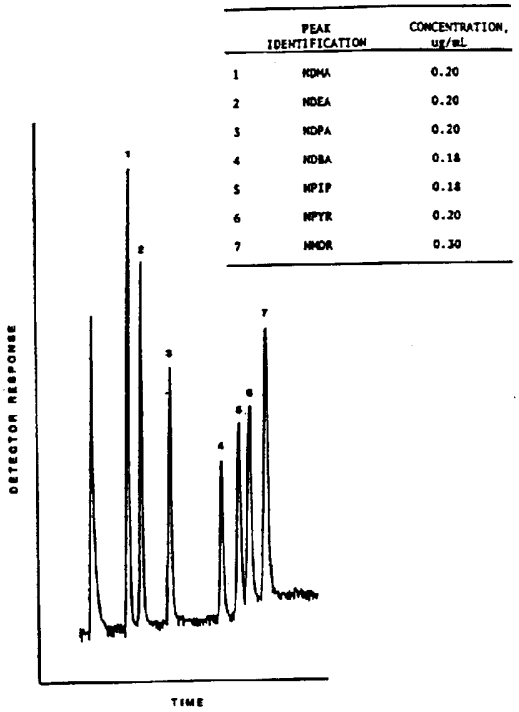
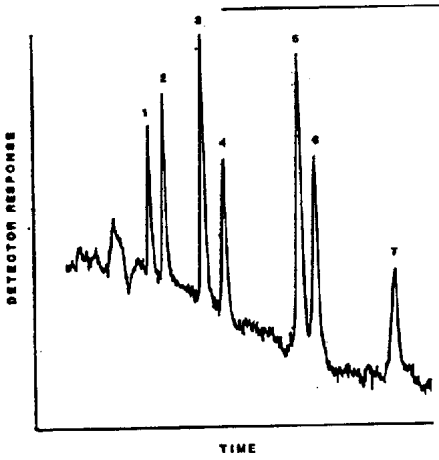


Figure 4.1. The detection limits of the analytical procedure.



	PEAK IDENTIFICATION	CONCENTRATION, ug/mL
1	NDMA	0.20
2	NDEA	0.20
3	NDPA	0.20
4	NDBA	0.18
5	NPIP	0.18
6	NPYR	0.20
7	NMOR	0.30

Figure 4.3.1. GC/TEA chromatogram of the analytes.



	PEAK IDENTIFICATION	CONCENTRATION ug/mL
1	NDBA	0.18
2	NDPA	0.20
3	NDEA	0.20
4	NPIP	0.18
5	NDMA	0.20
6	NMOR	0.30
7	NPYR	0.20

Figure 4.3.2. HPLC/TEA chromatogram of the analytes.

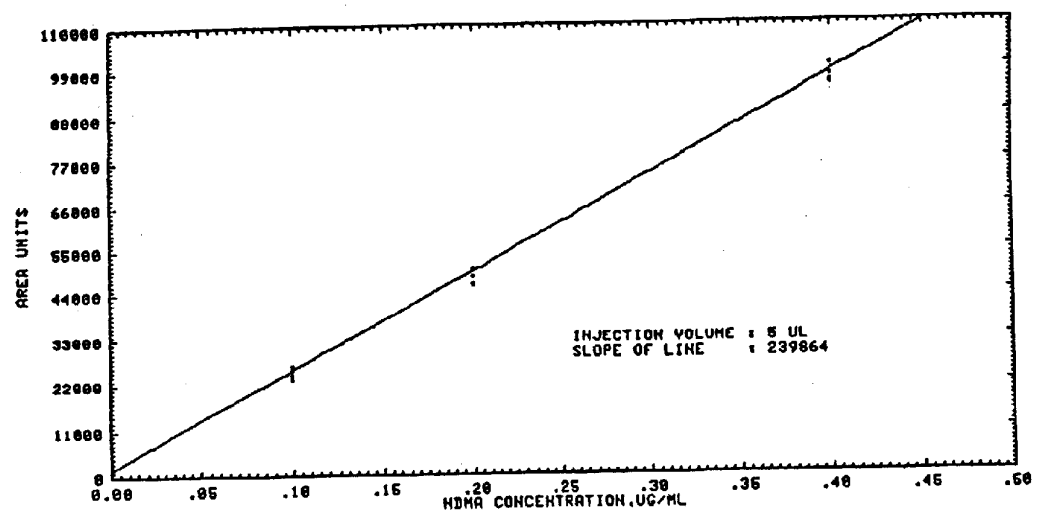


Figure 4.4.1. Calibration curve for N-nitrosodimethylamine.

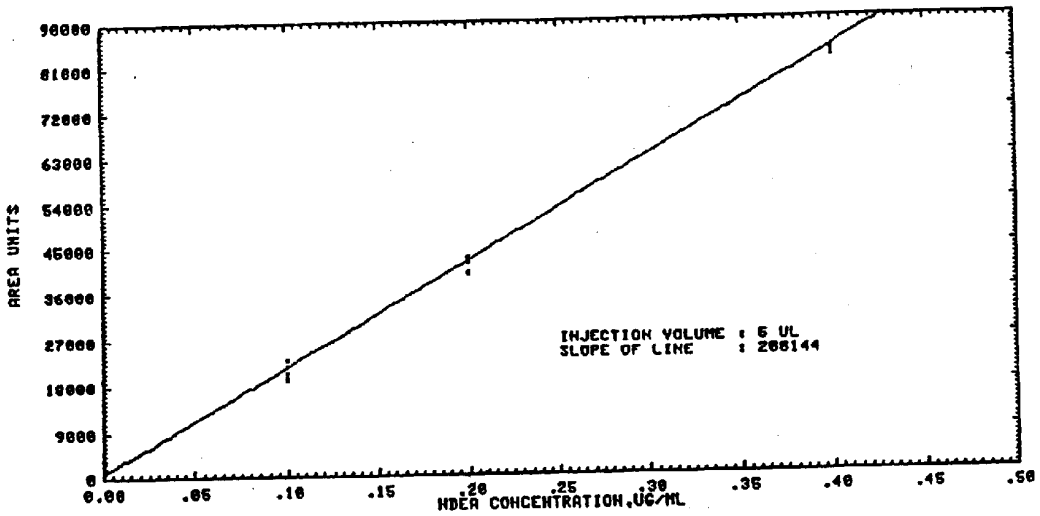


Figure 4.4.2. Calibration curve for N-nitrosodiethylamine.

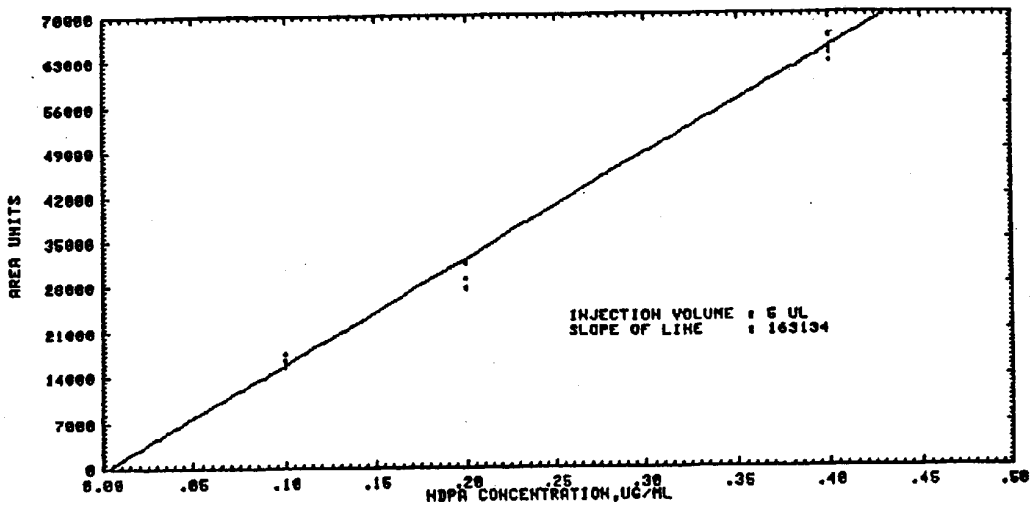


Figure 4.4.3. Calibration curve for N-nitrosodipropylamine.

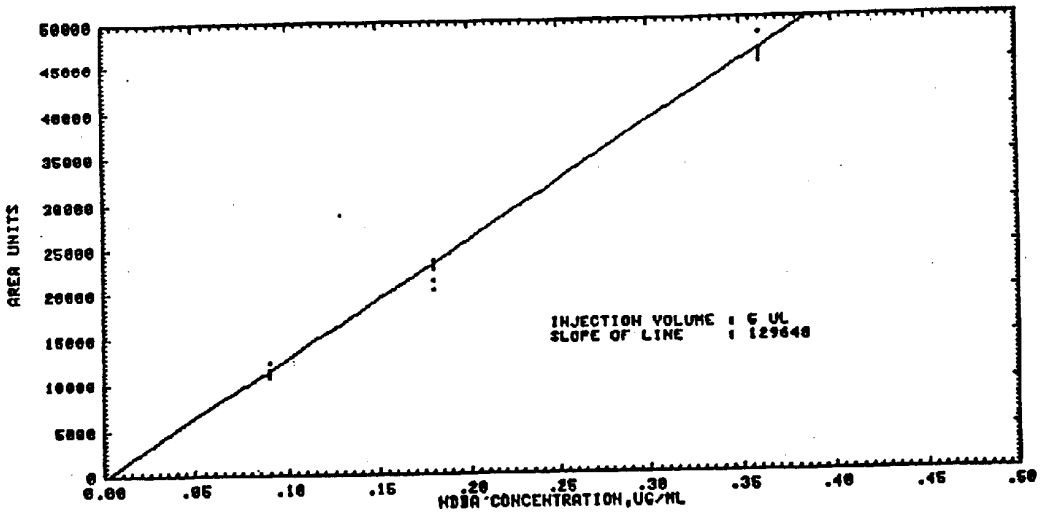


Figure 4.4.4. Calibration curve for N-nitrosodibutylamine.

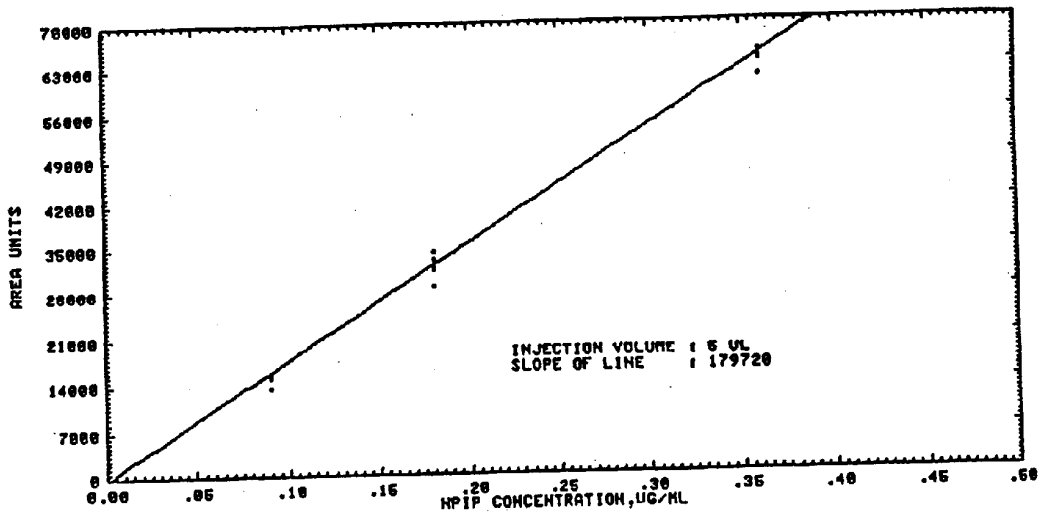


Figure 4.4.5. Calibration curve for N-nitrosopiperidine.

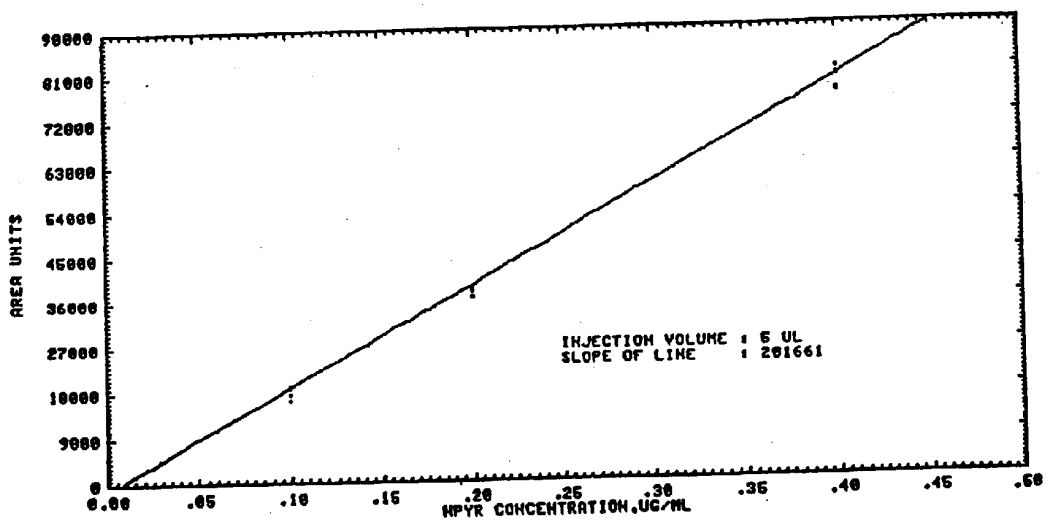


Figure 4.4.6. Calibration curve for N-nitrosopyrrolidine.

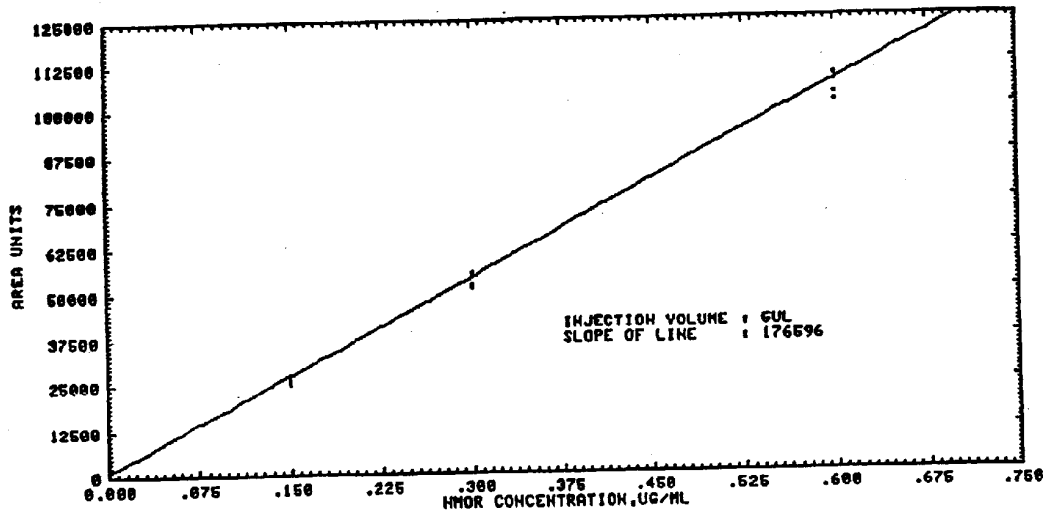


Figure 4.4.7. Calibration curve for N-nitrosomorpholine.

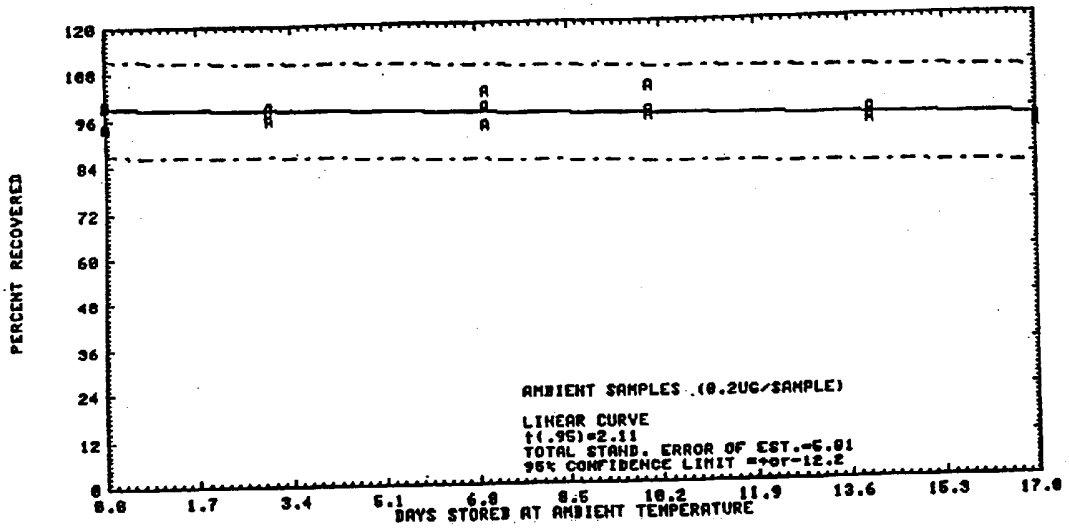


Figure 4.7.1. Ambient temperature storage test for N-nitrosodimethylamine.

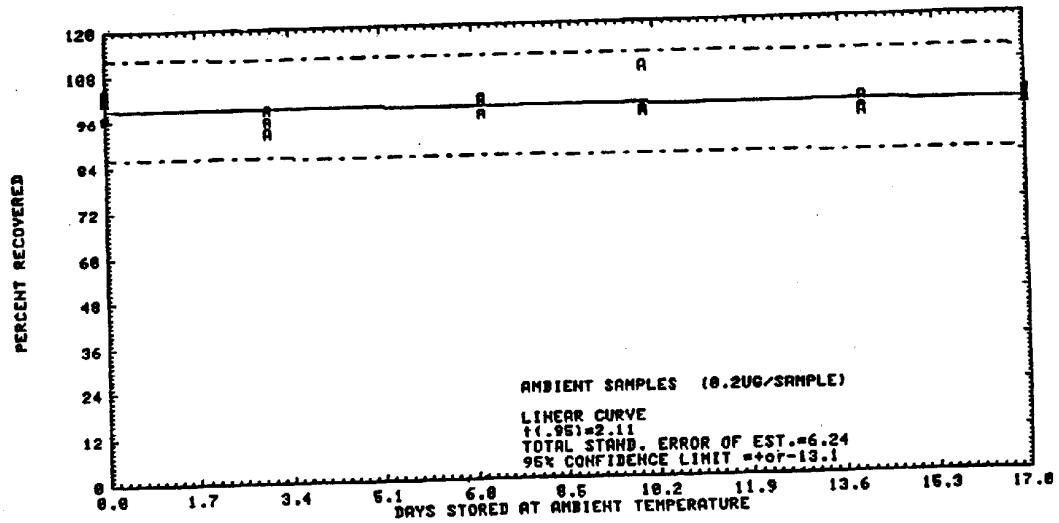


Figure 4.7.2. Ambient temperature storage test for N-nitrosodiethylamine.

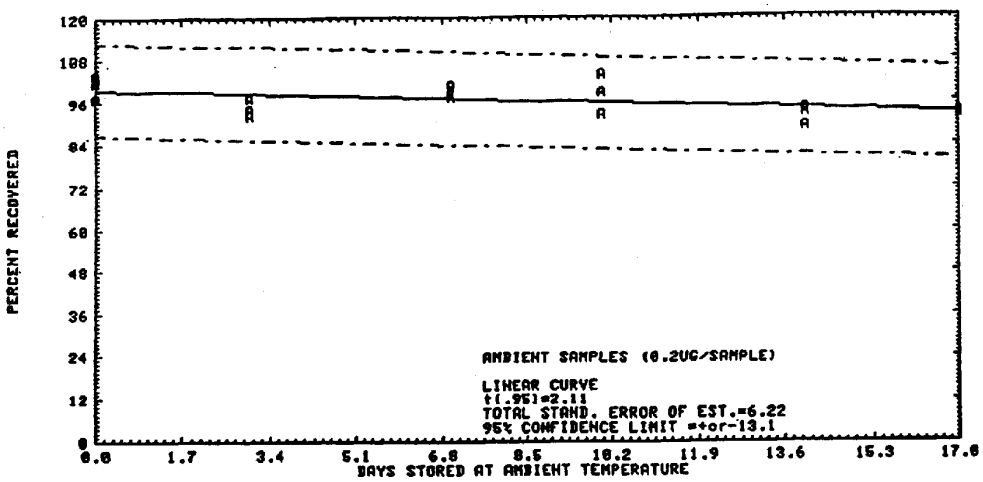


Figure 4.7.3. Ambient temperature storage test for N-nitrosodipropylamine.

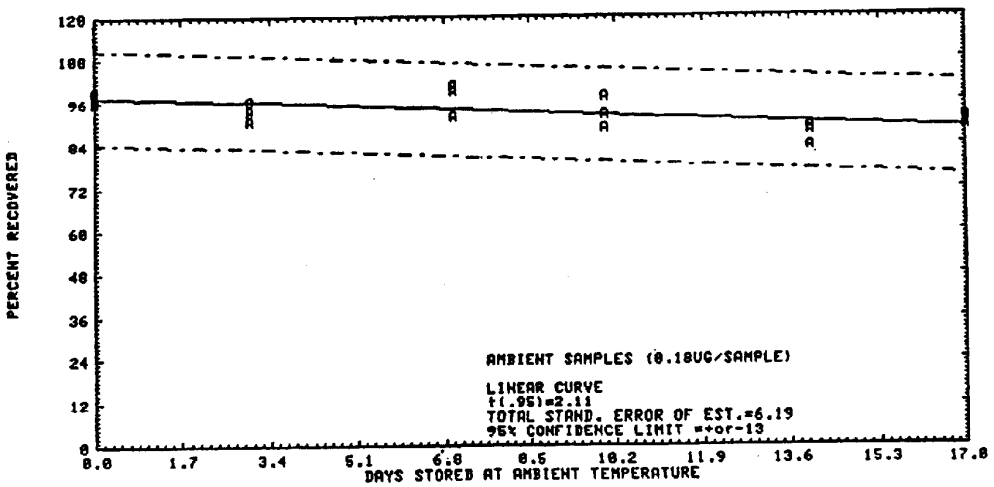


Figure 4.7.4. Ambient temperature storage test for N-nitrosodibutylamine.

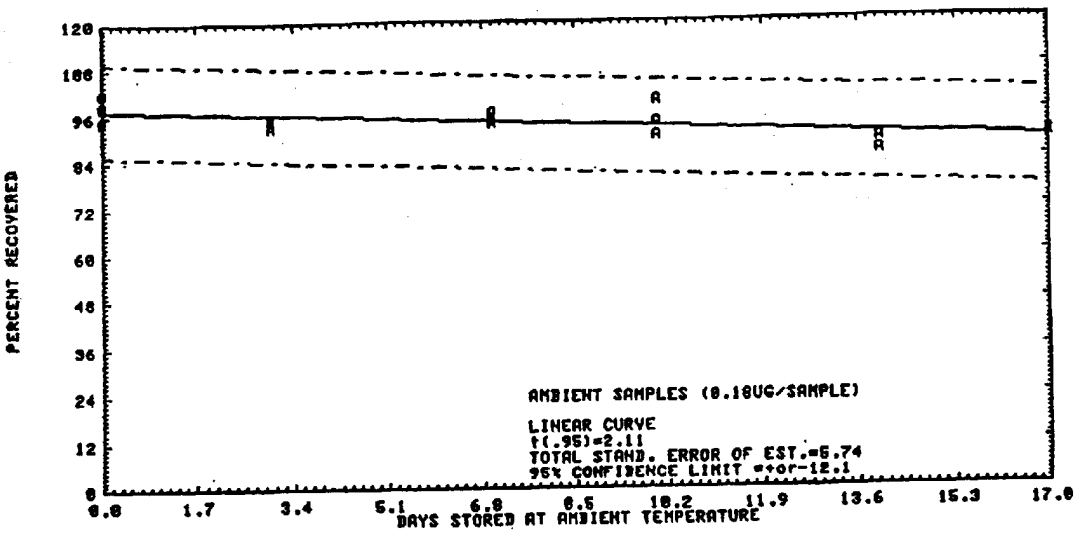


Figure 4.7.5. Ambient temperature storage test for N-nitrosopiperidine.

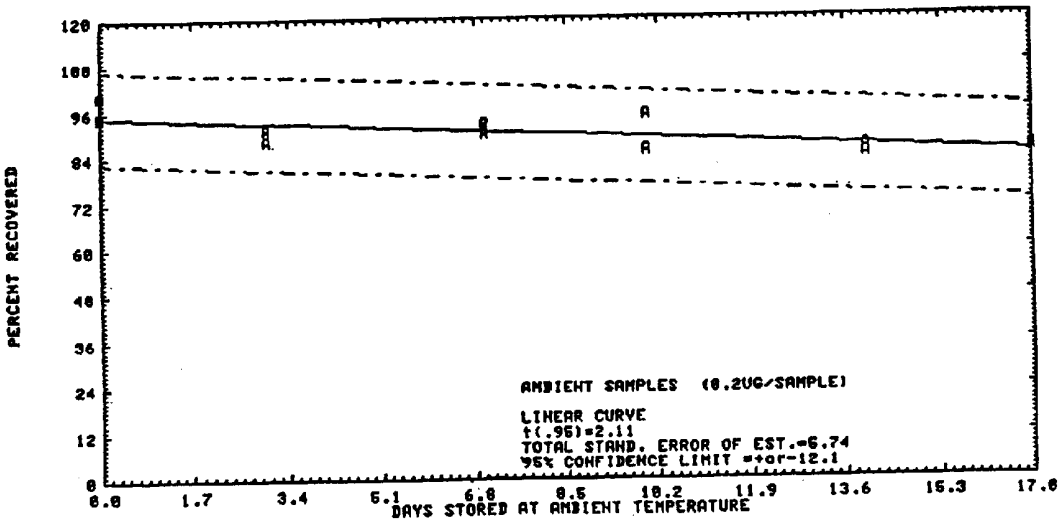


Figure 4.7.6. Ambient temperature storage test for N-nitrosopyrrolidine.

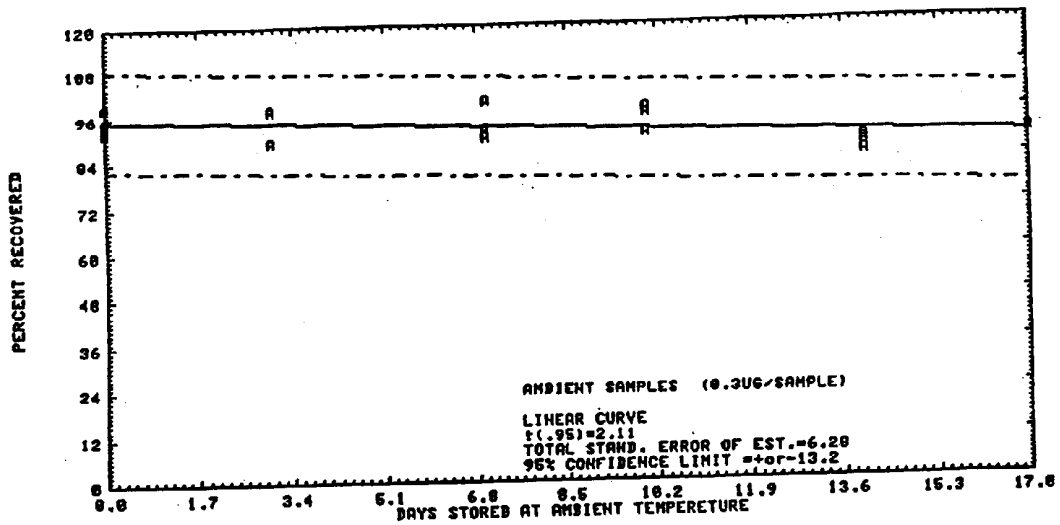


Figure 4.7.7. Ambient temperature storage test for N-nitrosomorpholine.

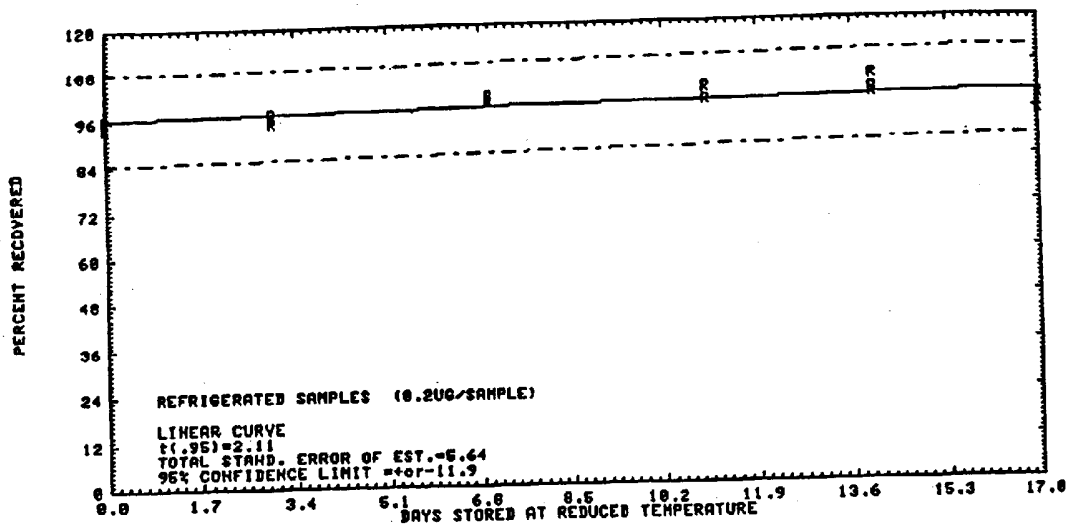


Figure 4.7.8. Reduced temperature storage test for N-nitrosodimethylamine.

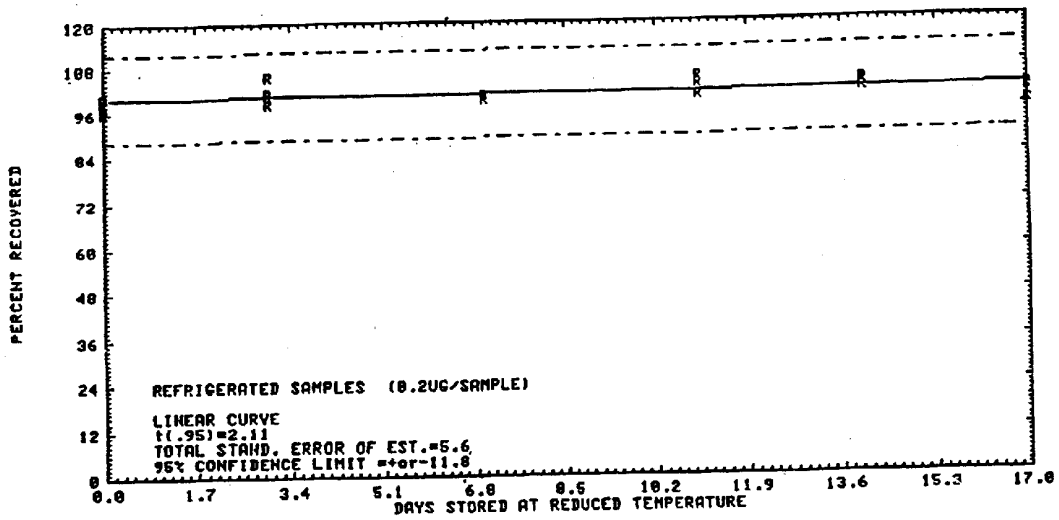


Figure 4.7.9. Reduced temperature storage test for N-nitrosodiethylamine.

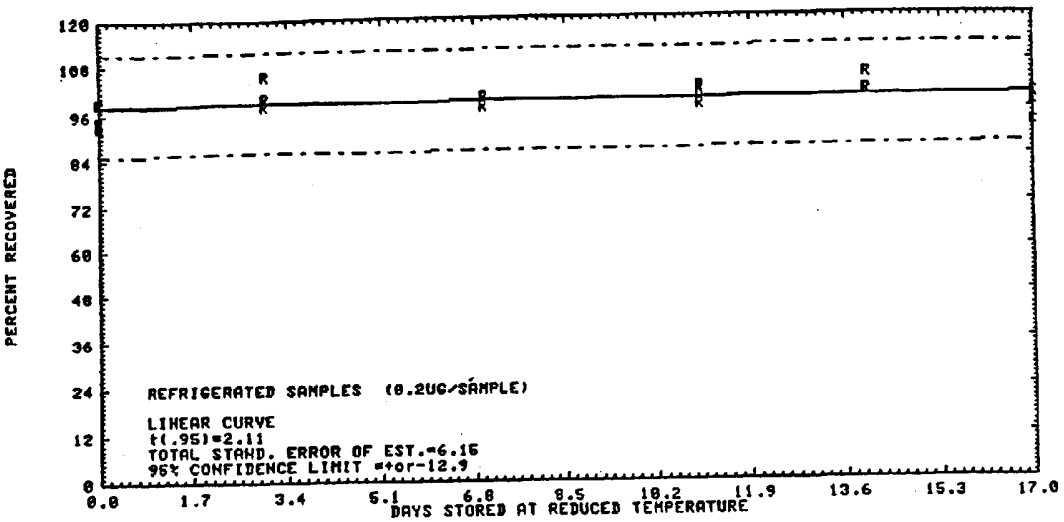


Figure 4.7.10. Reduced temperature storage test for N-nitrosodipropylamine.

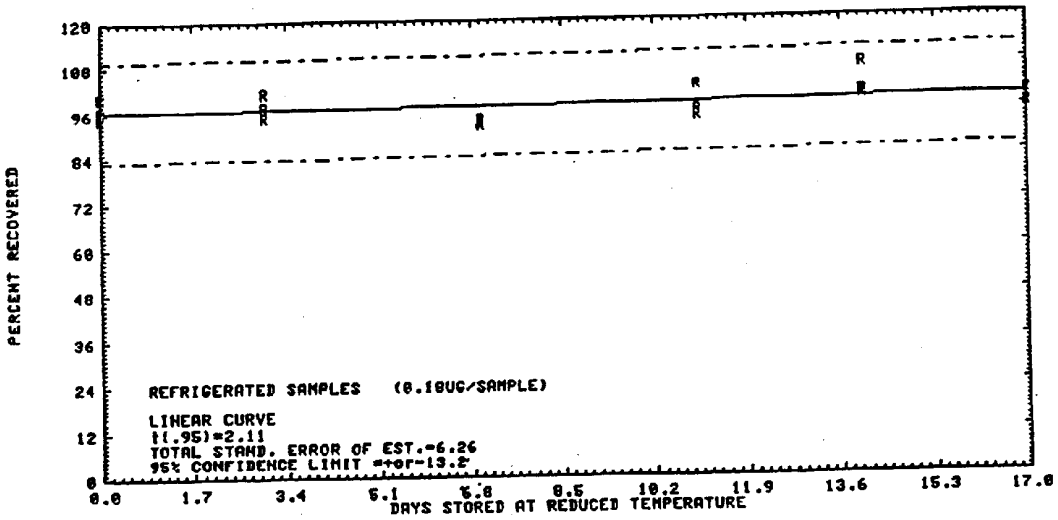


Figure 4.7.11. Reduced temperature storage test for N-nitrosodibutylamine.

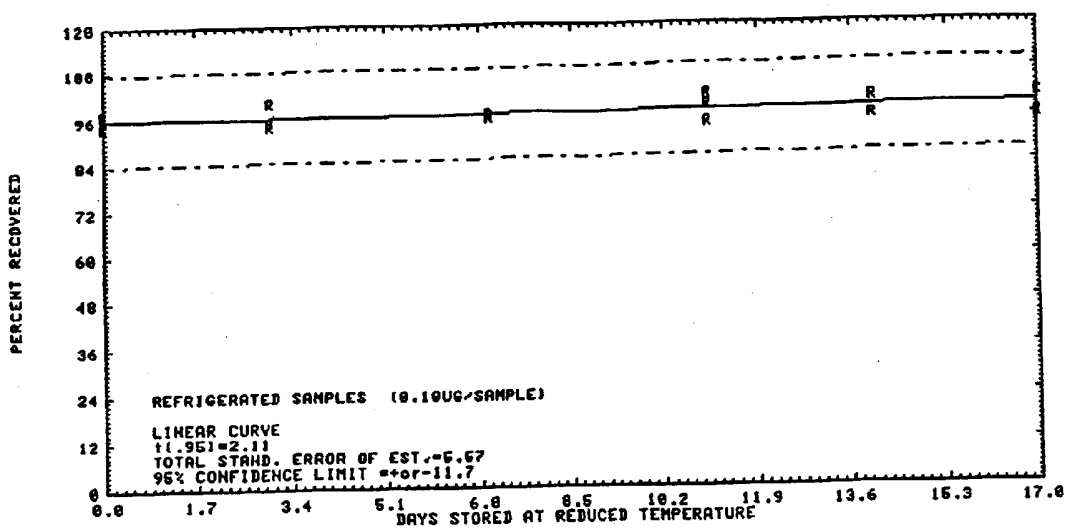


Figure 4.7.12. Reduced temperature storage test for N-nitrosopiperidine.

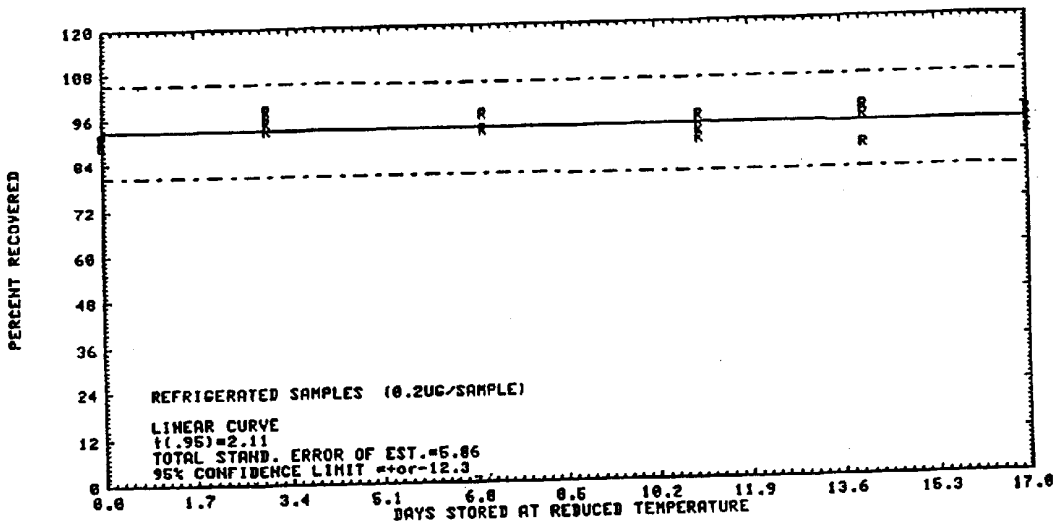


Figure 4.7.13. Reduced temperature storage test for N-nitrosopyrrolidine.

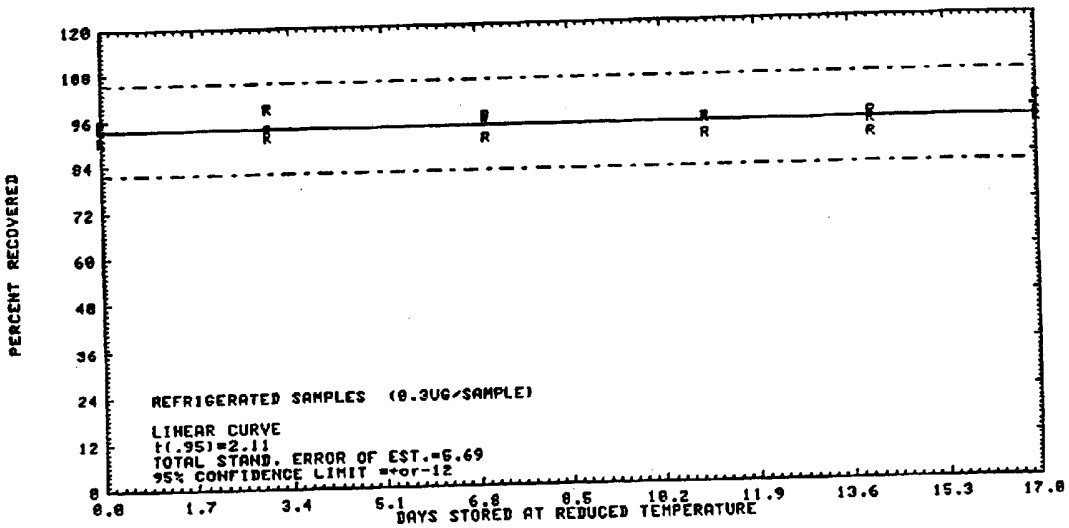


Figure 4.7.14. Reduced temperature storage test for N-nitrosomorpholine.

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