N-NITROSOMORPHOLINE

Method no.:	17
Matrix:	Air
Target concentration:	1.2 ppb (5.5 µg/m^3)
Procedure:	Samples are collected by drawing a known volume of air through two sampling tubes connected in series. The first tube contains Polar Partition and the second tube contains Florisil; both adsorbents are coated with ascorbic acid. Samples are desorbed with 75/25 (v/v) methylene chloride/methanol. Analysis is by gas chromatography using a chemiluminescence detector.
Recommended air volume and sampling rate:	40 L at 0.2 L/min
Detection limit of the overall procedure:	84 parts per trillion (ppt) (0.4 µg/m^3)
Reliable quantitation limit:	116 ppt (0.6 µg/m^3)
Standard error of estimate at the target concentration: (Section 4.6.)	6.0%
Special requirements:	The treated adsorbent tubes must be protected from light during and after sampling. The samples should be stored in a freezer.
Status of method:	A sampling and analytical method which has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch.

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1. General Discussion

   1.1. Background

      1.1.1. History of analysis

         Sample collection: N-Nitrosomorpholine (NMOR) vapors have been collected using impingers containing 1 N KOH as the trapping solution (Ref. 5.1.) and on Tenax GC cartridges (Ref. 5.2.).

         Analytical: Analytical procedures for NMOR are similar to those used for dimethylnitrosamine (DMN) and diethylnitrosamine (DENA). The separation is usually by gas chromatography and detection is by one or more of the following: mass spectrometry, Coulson Electrolytic Conductivity Detector, Hall Electrolytic Conductivity Detector, nitrogen selective alkali flame ionization detector and Thermal Energy Analyzer (TEA). (Refs. 5.3. and 5.4.) Liquid chromatographic procedures that utilize both ultraviolet and TEA detection systems have been reported. (Ref. 5.1.)

      1.1.2. Discussion concerning the sampling and analytical method

         This method recommends the use of two treated adsorbent tubes in series as an air sampling device for NMOR. The analytical separations are performed by gas chromatography and detection is accomplished using the TEA. The TEA detector was selected because it is both sensitive and selective for nitrosamines.

         An important requirement of any air sampling technique for nitrosamines is that the artifactual formation of the analyte on the sampling device from its precursors must be prevented. The sampler recommended for DENA (Florisil coated with Vitamin E) was found to be inadequate for the collection of NMOR because of artifact problems. Artifact formation experiments showed that Vitamin E did not prevent nitrosation from occurring in the presence of nitrogen oxides and the NMOR precursor amine morpholine. Not only did Florisil effectively collect (and thus concentrate) morpholine, Florisil coated with Vitamin E was an even more effective collector of morpholine than Florisil alone. Therefore, morpholine was available to react with nitrogen oxides to form NMOR. This reaction occurred before the nitrogen oxides could be removed by the Vitamin E. These artifact formation experiments involved drawing air containing nitrogen oxides through the sampler after it had received a loading of morpholine.

         Further experiments showed that Vitamin C coated Polar Partition (OV-275 on Chromosorb A) when placed ahead of Vitamin C coated Florisil prevented the artifactual formation of NMOR. When a coated Polar Partition tube was placed in series ahead of a coated Florisil tube, NMOR passed through the first tube and was collected on the second tube. Morpholine was not effectively collected on the first tube, and experiments showed that the artifactual formation of NMOR on the first tube did not occur. Also, experiments with the two tubes in series showed that even if morpholine did reach the second tube, artifactual formation of NMOR did not occur there since the nitrosating agent was removed by the first tube. Therefore, the sampler which was evaluated for this method consisted of two adsorbent tubes connected in series. The first contained Polar Partition and the second contained Florisil. The contents of each tube were coated with 10 mg of Vitamin C.

         The recommended NMOR air sampler is probably not suitable to sample DMN or DENA vapors because the device does not have the quantity of Florisil adsorbent that is necessary to collect and retain the more volatile nitrosamines. An air sampler with sufficient capacity for all three analytes could be assembled, however, it is unlikely that this device would be a universal air sampler for all nitrosamines because each analyte has unique properties that must be considered. It may be possible, when we have evaluated several samplers and nitrosamines, to develop a more general sampling device.

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

Acute: The LD_{50} for NMOR, administered by intraperitoneal injection to the rat, was 282 mg/kg. (Ref. 5.6.) The LD_{50}, following administration of NMOR with an esophageal probe to rats, was 320 mg/kg. (Ref. 5.7.) The agent was much more toxic when administered by intravenous injection - 3 the LD_{50} was 98 mg/kg for rats (Ref. 5.7.). The most important result of acute exposure to NMOR is extreme destruction of centrilobulor liver tissue. (Ref. 5.8.) The acute toxic effects normally develop within one or two days and a lethal dose usually kills the animal within a week. (Ref. 5.8.)

Chronic: NMOR has been reported to be carcinogenic to the European hamster (Ref. 5.9.), the mouse (Ref. 5.10.), the Syrian golden hamster (Ref. 5.11.), aquarium fish (Ref. 5.12.), to the guinea pig (Ref. 5.13.), and to the rat (Ref. 5.14.). The agent induced tumors in the lungs, liver, nasal cavity, trachea, and upper digestive tract following different modes of administration which included subcutaneous injection, oral ingestion, inhalation, skin painting, and intravenous injection. In all cases, animal survival decreased with increasing NMOR dosage and tumor yields of up to 100% were obtained.

NMOR was reported to be the most rapidly acting liver carcinogen of the 65 different nitrosamines administered to rats by Druckey, et al. When 16 rats were given 8 μg/kg (1/40 of the LD_{50}) NMOR daily in the drinking water, 14 animals died exclusively of liver cancer after an average tumor induction period (t_{50}) of 165 days. The average total dose which was applied up to the appearance of cancers in 50% of the animals (D_{50}) was 1.3 g/kg. In contrast to the oral administration, when 5 rats were given 10 mg/kg (1/10 of the LD_{50}) once per week by intravenous injection, 2 animals died of cancer of the nasal region and 1 of liver cancer. The D_{50} was 0.5 g/kg and the t_{50} was 440 days. Perhaps the reason for the apparent weaker carcinogenic effect following intravenous administration was that much lower doses were given in consideration of the higher toxicity (lower LD_{50}) of intravenous as compared to oral administration. (Ref. 5.7.)

Data regarding the toxicity of NMOR to humans are unavailable, however, the wide range of species that are subject to carcinogenesis by the agent indicates that humans may also be susceptible. While there is no direct evidence that exposure to nitrosamines leads to cancer in humans, indirect evidence obtained from studies that measured the relative metabolic rates of DMN by rat and human liver slices, indicate that man is probably about as sensitive to the carcinogenic action of DMN (and perhaps other nitrosamines) as is the rat. (Ref. 5.15.)

1.1.4. Operations where exposure occurs

NMOR is extensively used in cancer research facilities. Human exposure can occur when unchanged NMOR is excreted by the laboratory animals.

Many amines have been shown to be contaminated with the corresponding N-nitrosamine. (Ref. 5.16.) Exposure to NMOR can occur in operations where morpholine (the parent amine for NMOR) is used or produced. Further, if the amine is used as a chemical intermediate, NMOR can appear in the reaction product.

It has been shown that secondary and tertiary amines can react with oxides of nitrogen in the vapor phase to produce the corresponding nitrosamines. (Ref. 5.17.) Studies conducted in our laboratory confirm that morpholine is very easily nitrosated by nitrogen oxides and that ambient winter Salt Lake City air is sufficient to nitrosate the amine.

Potential exposure to NMOR exists whenever morpholine vapors are present because of the ease with which the agent can be nitrosated. Further, any tertiary amine which contains the morpholine moiety can be nitrosated to give NMOR. Industrial uses for morpholine include: as a rubber accelerator; a solvent; a reactant for organic synthesis; an additive to boiler water; in waxes and polishes; an optical brightener for detergents; a corrosion inhibitor and for preservation of book paper. (Ref. 5.18.)

The fairly common practice of the addition of steam to ambient winter air in order to increase the relative humidity of the air for its subsequent use in heated areas is an example of potential exposure to NMOR. Morpholine is a corrosion inhibitor that is

often added to boiler water and steam generated from such boiler water contains morpholine which can be nitrosated by the nitrogen oxides found in ambient air. (Ref. 5.19.)

NMOR has been reported to be present in the tire and rubber industry. The agent was found as an air pollutant in a tire chemical plant and an aircraft tire factory. NMOR, found in the chemical plant, was present in waste water (0.003 µg/g), a dirt scraping from a stairway (730 µg/g), a soil sample taken outside the plant (4.4 µg/g). Air samples taken in the chemical plant varied from 0.07 µg/m³ in the lunchroom to 5.1 µg/m³ near a diphenylnitrosamine reactor. The agent was found to be present in every site sampled at the tire factory. The lowest air concentration was 0.6 µg/m³ in the finishing and inspection area and the highest, 27 µg/m³ adjacent to a tire tread extruder. The source of the compound is unknown, however, morpholine levels of 230 and 42 µg/m³ were found at the tire factory and chemical plant, respectively. (Ref. 5.1.)

NMOR has been determined to be present in morpholine-nitrite salts used as vapor-phase corrosion inhibitors (Ref. 5.20.) and in certain hydraulic fluids. (Ref. 5.21.)

A non-occupational source of exposure to NMOR is the endogenous formation of the agent in the gastrointestinal tract. Morpholine has been shown to react with nitrite to form NMOR in the stomachs of Syrian hamsters. (Ref. 5.22.) The amine has been reported to react with human saliva to form NMOR. (Ref. 5.23.) Morpholine is used as a food additive, it is permitted in food intended for human consumption. (Ref. 5.24) Sources of dietary nitrite include the reduction of nitrate by microorganisms and nitrite cured food products. (Ref. 5.23.)

1.1.5. Number of workers that are exposed - unknown

1.1.6. Physical properties (Refs. 5.6. and 5.7.)

synonyms:	Morpholine, N-nitroso; 4-nitrosomorpholine; n-nitrosomorpholin (German)
CAS no.:	59-89-2
molecular formula:	$C_4H_8N_2O_2$ (MOR-NO)
molecular structure:	Figure 1.1.6.
molecular weight:	116.14
boiling point:	96°C (6 mm Hg)
melting point:	29°C

absorp. spec.:

λ max	log ε
237	3.90
346	1.93

(in water)

solubility in water: unlimited
distribution coefficient (concentration in hexane to concentration in pH 7.4 phosphate buffer): 0.039
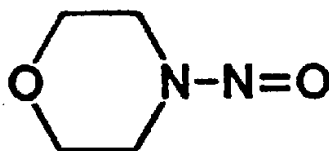

Figure 1.1.6. Molecular structure of N-nitrosomorpholine

1.2. Limit defining parameters

1.2.1. Detection limit of the analytical procedure

The detection limit of the analytical procedure is 80 pg (0.016 µg/mL × 5 µL injection volume) per injection. This is the amount of analyte which will give a peak whose height is about five times the amplitude of the baseline noise. (Section 4.1.)

Note: OSHA no longer uses or supports this method (December 2019).

1.2.2. Detection limit of the overall procedure

The detection limit of the overall procedure is 0.016 µg/ sample (84 ppt or 0.4 µg/m^3). This is the amount of NMOR spiked on the treated adsorbent tubes which allows recovery of an amount of the analyte equivalent to the detection limit of the analytical procedure. (Section 4.2.)

1.2.3. Reliable quantitation limit

The reliable quantitation limit for the analytical method was 0.022 µg/sample (116 ppt or 0.6 µg/m^3). This is the smallest amount of analyte which can be quantified within the requirements of 75% recovery and 95% confidence limits of ±25%. The reliable quantitation limit was determined by spiking the sampling device with the analyte. The average recovery from treated Florisil tubes was 96.8% and the 95% confidence interval limits were ±17.2%. The average recovery from treated Polar Partition tubes was 98.7% and the 95% confidence interval was ±18.5%. (Section 4.3.)

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 procedure over a concentration range representing 0.5 to 2 times the target concentration based on the recommended air volume is 44085 area units (HP-5840A) µg/mL. The sensitivity is determined by the slope of the calibration curve. (Figure 4.4.1.) The sensitivity will vary somewhat with the particular instrument used in the analysis. A representative chromatogram is shown in Figure 4.4.2.

1.2.5. Recovery

The average recovery over the range of 0.5 to 2 times the target concentration was 96.3% for treated Florisil tubes and 96.7% for treated Polar Partition tubes. (Section 4.5.) The recovery remained above 81% during the 19-day ambient temperature storage period. (Section 4.6.) The recovery of analyte from the collection medium must be 75% or greater.

1.2.6. Precision of the analytical method

The pooled coefficient of variation obtained from replicate determinations of analytical standards at 0.5, 1, and 2 times the target concentration was 0.028. (Section 4.7.)

1.2.7. Precision of the overall procedure

The overall procedure must provide results at the target concentration that are ±25% or better at the 95% confidence level. The 95% confidence limits for the 19 day ambient temperature storage test are ±12.6%. (Section 4.6.). This includes an additional ±5% for sampling error.

1.2.8. Reproducibility

The analytical method was shown to be reproducible by the analysis of liquid-spiked samples by a second chemist who was not associated with this evaluation. The average recovery from treated Polar Partition tubes spiked with 0.22 µg of NMOR was 97.9%. The average recovery from treated Florisil tubes spiked with 0.11 µg of NMOR was 100.3%. (Section 4.8.)

1.3. Advantages

1.3.1. The sampling procedure is convenient.

1.3.2. The significance of artifactual formation of NMOR upon the sampling device has been eliminated through pretreatment of the air sampler.

Note: OSHA no longer uses or supports this method (December 2019).

1.3.3.   The analytical procedure is quick, sensitive, and reproducible.

1.3.4.   Reanalysis of the samples is possible.

1.3.5.   It may be possible to determine other nitrosamines simultaneously.

1.3.6.   The effects of potential interferences are reduced through the use of a selective detector (the TEA) and can be further reduced by proper selection of GC parameters.

1.3.7.   The air sampler is composed of commercially available materials.

1.4.   Disadvantages

1.4.1.   At this time the sampling method has not been field tested.

1.4.2.   The cost of the TEA may be prohibitive to small laboratories.

2.   Sampling Procedure

2.1.   Apparatus

2.1.1.   An approved and calibrated personal sampling pump whose flow can be determined to ±5% at the recommended flow rate.

2.1.2.   Florisil and Polar Partition adsorbent tubes: Glass tubes, 6-mm o.d., 4-mm i.d., 7-cm length, containing 100-mg front and 50-mg rear (separated by a 2-mm portion of urethane foam or silylated glass wool) sections of 20/40 mesh Florisil and Polar Partition adsorbent. SKC Inc. Catalog Nos. 226-39 and 226-26 or equivalent. Each tube is pretreated with 10 mg L-(+)-ascorbic acid. Instructions for the preparation of the coated sampling tubes are given in Section 4.9.

2.2.   Reagents

None required.

2.3.   Sampling technique

2.3.1.   The air sampler is composed of a treated Polar Partition tube followed by a treated Florisil tube in series. The tubes are easily connected with an end cap that has been modified by cutting off the closed portion.

2.3.2.   Connect the air sampler to the sampling pump with flexible tubing. The 50-mg section of each tube should be positioned toward the sampling pump. Cover each tube of the air sampler with masking tape or other suitable material to prevent light from reaching the adsorbent.

2.3.3.   The air sampler should be placed in a vertical position during sampling to minimize channeling.

2.3.4.   Sampled air should not pass through any hose or tubing before entering the sampling device.

2.3.5.   Immediately after sampling, separate the air sampler into its component tubes, identify each tube as front or backup and seal each tube with plastic end caps. Also, wrap each sample end to end with official OSHA seals.

2.3.6.   With each batch of samples, submit at least one blank tube of each adsorbent material from the same lot used for samples. These tubes should be subjected to exactly the same handling as the samples (seal, transport) except that no air is drawn through them.

2.3.7.   Transport the samples (and corresponding paperwork) to the lab for analysis.

Note: OSHA no longer uses or supports this method (December 2019).

2.3.8. If bulk samples are submitted for analysis, they should be transported in glass containers with Teflon-lined caps. The samples must be kept from light. These samples must not be put in the same container used for the treated adsorbent tubes.

2.4. Sampler capacity

Breakthrough, from the Polar Partition tube to the front section of the Florisil tube, was 75% after about 40 L of air containing target level concentrations of NMOR at 80% relative humidity and 29°C were sampled at 0.2 L/min.

Breakthrough from the front to the rear section of the Florisil tube was 5% after about 85 L of air containing twice the target level of NMOR at 80% relative humidity and 21°C were sampled at 0.2 L/min.

The breakthrough volume remained constant when the flow rate was increased from 0.2 to 0.5 L /min. The pressure drop across the air sampler was less than 1 in. of mercury at 0.5 L/min. If larger air volumes or shorter sampling times are desired, the flow rate can be increased to 0.5 L/min. The air volume should be no greater than 85 L. The possibility of interferences will increase as the air volume sampled is increased.

2.5. Desorption efficiency

2.5.1. The average desorption from L-(+)-ascorbic acid treated Polar Partition tubes spiked with 112, 224, and 448 ng of NMOR was 96.7% (Section 4.5.).

2.5.2. The average desorption from L-(+)-ascorbic acid treated Florisil tubes spiked with 112, 224, 448 ng of NMOR was 96.3% (Section 4.5.).

2.5.3. The spiked tubes represent an air concentration range of 2.8 to 11.3 μg/m^3 (0.6 to 2.4 ppb) based on the recommended air volume.

2.5.4. The desorption efficiency for NMOR may vary somewhat from one laboratory to another and also from one lot of L-(+)-ascorbic acid treated Polar Partition and Florisil tubes to another. Therefore, it is necessary for each laboratory to determine the desorption efficiency for each lot of treated adsorbent tubes.

2.6. Recommended air volume and sampling rate

2.6.1. The recommended air volume is 40 L.

2.6.2. The recommended sampling rate is 0.2 L/min.

2.7. Interferences

2.7.1. Since it is possible that the precursors of NMOR, which are morpholine, and various nitrosating agents (oxides of nitrogen, nitrites, etc.), are present in the environment, it is conceivable that NMOR may be formed upon the sampling device and not be present in the sampled air. Laboratory experiments indicate that it is possible to form NMOR from its precursors on an untreated air sampler. Further experiments show that when the tubes are treated with 10 mg of L-(+)-ascorbic acid, the formation of NMOR does not take place.

2.7.2. At the present time, it is unknown if any compound would severely interfere with the collection or retention of NMOR on treated Polar Partition and Florisil tubes. In general, the presence of other compounds will reduce the breakthrough volume for a particular compound.

2.7.3. Any compound which is suspected of interfering with the collection or analysis should be listed on the sampling data sheet.

2.7.4. Light will decompose NMOR (Ref. 5.7.). The air sampler must be protected from light during and after sampling.

2.8. Safety precautions

2.8.1. Observe due care when working with the sharp ends of the air sampler.

Note: OSHA no longer uses or supports this method (December 2019).

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

      2.8.3.   Follow all safety practices that apply to the work area being sampled.

3.   Analytical Procedure

    3.1.   Apparatus

      3.1.1.   A gas chromatograph interfaced to a Thermal Energy Analyzer.

      3.1.2.   A GC column capable of resolving NMOR from the desorption solvent and potential interferences. The column used in this work was a 10-ft × 1/8-in. stainless steel column containing 10% Carbowax 20M with TPA on 80/100 mesh Chromosorb W AW.

      3.1.3.   An electronic integrator or other suitable method to measure peak area.

      3.1.4.   An analytical balance capable of accurately weighing to 5 decimal places.

      3.1.5.   Vials, 2-mL vials with Teflon-lined caps.

      3.1.6.   Microliter syringes, 5-µL syringe for sample injections and other convenient sizes.

      3.1.7.   Pipets, convenient sizes for diluting standards. A 1-mL dispenser pipet for the desorbing solvent.

      3.1.8.   Volumetric flasks, convenient sizes for diluting standards.

      3.1.9.   Dewar flasks, convenient sizes for liquid nitrogen.

      3.1.10. Heating tape, high temperature, heavily insulated.

      3.1.11. Variable voltage transformer.

    3.2.   Reagents

      3.2.1.   NMOR, authentic primary standard, 98% minimum.

      3.2.2.   Methyl alcohol, chromatographic grade.

      3.2.3.   Methylene chloride, chromatographic grade.

      3.2.4.   Isopropyl alcohol, chromatographic grade.

      3.2.5.   Ethyl alcohol, U.S.P., 95%.

      3.2.6.   Gases, purified GC grade helium and medical grade oxygen.

      3.2.7.   Nitrogen, liquid.

    3.3.   Sample preparation

      3.3.1.   The status of the OSHA seal on each sample is noted and recorded as intact, broken, or none.

      3.3.2.   The field and laboratory identification numbers on each sample are checked against those on the sample identification sheets.

      3.3.3.   Suitable precautions must be undertaken to prevent the exposure of the samples to light. NMOR will photodecompose easily.

      3.3.4.   The contents of the treated Polar Partition (front) tube (solid sorbent, glass wool and urethane plugs) are added to a 2-mL vial. The front glass wool plug and the 100-mg section of the treated Florisil (rear) tube are added to a second 2-mL vial. The

Note: OSHA no longer uses or supports this method (December 2019).

remaining 50 mg of treated Florisil, the glass wool and urethane plug are added to a third vial.

3.3.5. Analysis of the third vial will indicate if breakthrough has occurred. If 20% or more of the total NMOR found on the air sampler is present in the third vial, then breakthrough has occurred and an appropriate comment is made on the analysis sheet.

3.3.6. Each vial is desorbed with 1.0 mL of desorbing solution. The desorbing solution is composed of 75 parts methylene chloride and 25 parts methyl alcohol by volume.

3.3.7. The vials are sealed immediately with Teflon-lined caps and allowed to desorb for 30 min with intermittent shaking.

3.4. Standard preparation

3.4.1. Stock standards are prepared by diluting a weighed amount of NMOR with isopropyl alcohol. The stock standard is diluted to the working range with isopropyl alcohol (See Section 3.8. Safety Precautions).

3.4.2. A solution composed of 0.22 µg/mL NMOR in isopropyl alcohol equals an air concentration of 5.5 µg/m^3 for a 40-L air sample desorbed with 1.0 mL of desorbing solution. This amount is not corrected for desorption efficiency.

3.4.3. Standards are stored in dark bottles under refrigeration.

3.5. Analysis

3.5.1. GC conditions

helium (carrier gas) flow rate:	30 mL/min
injector temperature:	205°C
column temperature:	205°C
TEA transfer line temperature:	210°C
elution time:	2.5 min

3.5.2. TEA conditions

oxygen pressure:	9 psi
GC pyrolyzer furnace temperature:	475°C
coarse zero:	high
calibrate:	0.0
attenuator:	4
cold trap temperature:	-130°C (ethyl alcohol and liquid nitrogen)

Complete instructions for the TEA are found in its manual.

3.5.3. The transfer line between the GC and TEA must be maintained at about 210°C with a heating tape. The temperature is controlled with a variable voltage transformer.

3.5.4. Chromatogram (Section 4.4.)

3.5.5. Peak areas are measured by an electronic integrator or other suitable means.

3.5.6. An external standard procedure is used to prepare a calibration curve from the analysis of at least three different standard solutions. The calibration curve is prepared daily. The integrator is calibrated to report results in micrograms per milliliter.

3.5.7. Bracket the samples with analytical standards.

3.6. Interferences

3.6.1. The TEA is a highly selective detector for N-nitrosamines but false positive results have been reported. (Ref. 5.25.) The sample is separated into its components by gas or liquid chromatography. The N-nitroso compound, in the carrier gas of a GC

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or the mobile phase of an HPLC, enters a flash catalytic heater or pyrolyzer where the bonds between the two nitrogen atoms (N-NO) are ruptured forming nitrosyl radicals (•NO). Organic compounds, solvent and fragmentation products are collected in a cold trap.

The nitrosyl radicals in the carrier gas are then reacted with ozone, under vacuum, to form electronically excited nitrogen dioxide (NO_2^*). The excited nitrogen dioxide quickly decays to its ground state emitting light at a characteristic wavelength, which is measured by a photomultiplier tube.

Any compound which will form the nitrosyl radical will elicit a response from the TEA. In addition to N-nitroso compounds, the TEA will respond to organic and inorganic nitrites, some nitrates, certain C-nitroso compounds and certain C-nitro compounds.

Any compound that has the same GC column retention time as NMOR and has a TEA response is an interference.

3.6.2.   GC parameters may be changed to circumvent most interferences.

3.6.3.   Retention time on a single GC column is not proof of chemical identity. Samples should be confirmed by GC/MS or other suitable means when required.

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 the calibration curve. The peak area, for each standard, is compared to its concentration in µg/mL and the equation for the best straight line through the data points is determined by linear regression.

3.7.2.   The concentration in µg/mL for a sample is determined by comparing the area of a particular sample to the calibration curve.

3.7.3.   The result obtained from the analysis of each vial is corrected by the appropriate desorption efficiency and then the corrected results from the two tubes that compose a particular air sample are added together.

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

$$NMOR, \mu g/m^3 = [(1000)(A)(B)]/C$$

where   A = µg/mL from Section 3.7.3.
B = desorption volume
C = air volume in liters

3.7.5.   To convert the results from Section 3.7.4. in µg/m³ to parts per billion the following relationship is used:

$$NMOR, ppb = [(\mu g/m^3)(24.46)]/116.1$$

where   µg/m³ = result from Section 3.7.4.
24.46 = molar volume at 25°C and 760 mm Hg
116.1 = molecular weight of NMOR

3.8.   Safety precautions

3.8.1.   NMOR is an extremely potent animal carcinogen and utmost care must be exercised when working with this compound.

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.   Be certain that the TEA exhaust is connected to a fume hood.

4. Backup Data

4.1. Detection limit of the analytical procedure

Figures 4.1.1., 4.1.2., and 4.1.3. were used to determine the detection limit of the analytical procedure.

4.2. Detection limit of the overall procedure

The data in Table 4.2. are graphically represented in Figures 4.2.1. and 4.2.2. in order to determine the detection limit of the overall procedure.

Table 4.2
Detection Limit Data

µg NMOR spiked on treated adsorbent tubes	avg. µg NMOR recovered from treated Florisil tubes	avg. µg NMOR recovered from treated Polar Partition tubes
0.110	0.104	0.108
0.0450	0.0446	0.0437
0.0220	0.0213	0.0217
0.0160	0.0159	0.0170

4.3. Reliable quantitation limit

Treated adsorbent tubes were each spiked with 0.022 µg of NMOR and the recoveries were as follows:

Table 4.3.
Reliable Quantitation Limit Data

trial	% recovery from treated Florisil tubes	% recovery from treated Polar Partition tubes
1	102.3	100.0
2	102.3	96.2
3	88.6	106.7
4	98.9	88.9
5	92.1	101.8
$\bar{X}$	96.8	98.7
SD	6.2	6.7
1.96(SD)	12.2	13.1

4.4. Sensitivity

A typical calibration curve is shown in Figure 4.4.1. The slope of the curve indicates the sensitivity of the method.

A representative chromatogram is shown in Figure 4.4.2.

4.5. Desorption efficiency

The following data represent the recovery of NMOR spiked on treated Polar Partition and on treated Florisil adsorption tubes. The desorption study for the Florisil tubes was conducted with the reference (B) portion removed.

Note: OSHA no longer uses or supports this method (December 2019).

Table 4.5.
Desorption Efficiencies from Two Adsorbents

x target conc. ng/sample	Polar Partition			Florisil		
	0.5x 112	1x 224	2x 448	0.5x 112	1x 224	2x 448
desorption efficiency, %	96.6	91.8	99.1	91.2	97.2	95.6
	98.3	100.0	93.3	100.4	100.5	101.1
	96.5	93.9	100.0	91.7	95.5	98.8
	101.0	98.0	88.7	93.9	99.9	87.7
	99.0	98.0	96.4	93.7	94.5	93.5
	99.2	95.2	96.2	98.5	99.2	99.7
$\bar{X}$	98.4	96.2	95.6	94.9	97.8	96.1
$\bar{\bar{X}}$	Polar Partition = 96.7			Florisil = 96.3		

4.6.   Storage

Samples were generated by the liquid injection of NMOR on untreated polar partition tubes containing only 50 mg of adsorbent. The tubes were allowed to equilibrate overnight and then were placed in front of the treated air sampler. Forty liters of air at 80% relative humidity and 20°C were drawn through the sampling train. The NMOR on the Polar Partition tube was desorbed by the humid air and was deposited on the air sampler. Studies conducted at ambient relative humidity and temperature indicated that desorption was essentially complete after 25 L of air had passed through the Polar Partition tube (Figure 4.6.1.). In these studies, the spiked polar partition tube was connected directly to the TEA. The data in Table 4.6. represent the effects of storage at ambient (21-26°C) and reduced (-20°C) temperatures on NMOR collected using treated adsorption tubes. The recoveries are not corrected for desorption efficiency. A graphical representation of the data may be found in Figures 4.6.2. and 4.6.3.

Table 4.6
Storage Tests

storage time (days)	% recovery					
	(refrigerated)			(ambient)		
0	97.3	98.0	101.4	97.3	91.8	99.1
3	92.5	97.5	95.0	101.4	93.6	93.6
6	97.7	97.3	98.6			
7				92.0	92.3	90.0
10	100.5	99.8	98.9	85.4	93.0	89.5
14	98.0	97.7	100.0	90.7	81.8	83.4
19				80.2	82.5	81.4
20	94.1	104.5	100.4			

4.7.   Precision of the analytical method

These data represent multiple injections of standard solutions. The injection volume was 5 µL and the concentrations of the standards were 0.11, 0.22, and 0.45 µg/mL. Peaks were integrated by a Hewlett-Packard 5840A Gas Chromatograph.

Note: OSHA no longer uses or supports this method (December 2019).

Table 4.7.
Precision of the Analytical Method

x target conc.	0.5x	1x	2x
pg/injection	550	1100	2250
area counts	5117	9900	19830
	5017	10210	20530
	5017	10170	20490
	4842	10070	19490
	5072	10070	19210
	4918	9680	20280
	4742	9582	19530
	4788	9582	20008
	5371	9494	20450
$\overline{X}$	4987.11	9853.78	19979.78
SD	193.26	290.60	491.46
CV	0.0388	0.0295	0.0245

$\overline{CV}$ = 0.0282

4.8.   Reproducibility

Three treated Polar Partition and three treated Florisil tubes were spiked (liquid injection) with 0.22 and 0.11 µg NMOR. These samples were analyzed by a chemist unassociated with the development of this procedure. The results are shown below.

Table 4.8.
Reproducibility

	Polar Partition	Florisil
µg/sample	0.22	0.11
% NMOR	96.4	95.0
recovered	105.5	112.5
	91.8	93.3
$\overline{X}$	97.9	100.3

4.9.   Sampling tube preparation

4.9.1.   Reagents

Methyl alcohol, chromatographic grade.

L-(+)-Ascorbic acid (Vitamin C), USP Grade, 98% min. Prepare a solution containing 40 mg/mL in methyl alcohol. Store under refrigeration in a dark bottle.

4.9.2.   Technique

Each adsorbent tube is treated with 10 mg of L-(+)-ascorbic acid. This is accomplished by first breaking open both ends of each tube and adding 250 µL of the L-(+)-ascorbic acid solution. Both sections of the tube should be uniformly wet by the solution. The methyl alcohol is allowed to evaporate and then both ends of the tubes are sealed with the plastic end caps supplied with the tubes. The tubes should be stored in a freezer with suitable precautions taken to prevent their contamination. The tubes are stable, when stored in a freezer, for at least a month.

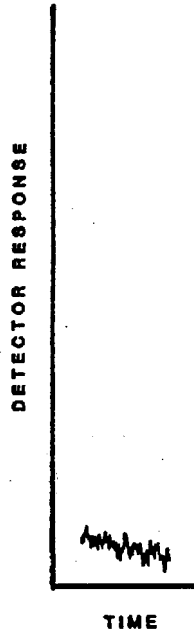Note: OSHA no longer uses or supports this method (December 2019).

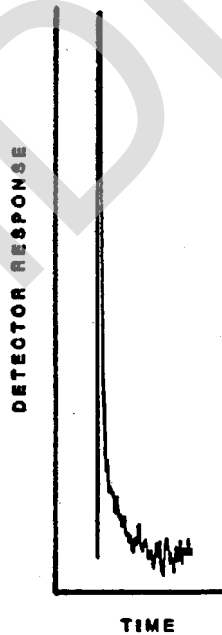Figure 4.1.1. Typical TEA detector noise.

Figure 4.1.2. A chromatogram of the desorption solvent.

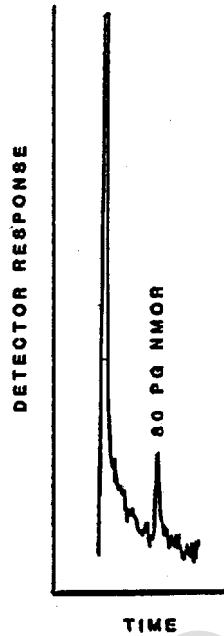Note: OSHA no longer uses or supports this method (December 2019).

Figure 4.1.3. Detection limit of the analytical procedure for N-nitrosomorpholine.
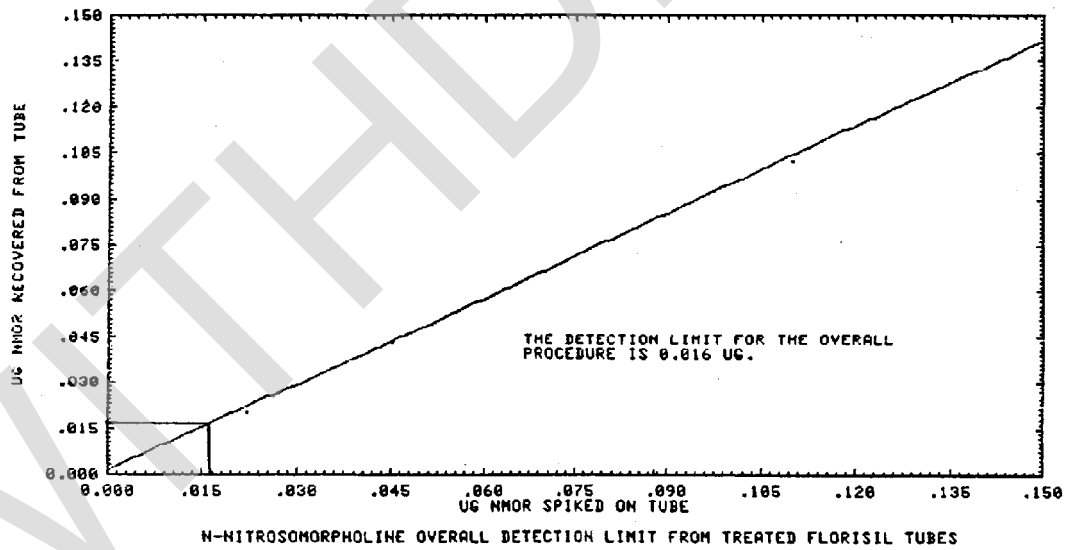

N-NITROSOMORPHOLINE OVERALL DETECTION LIMIT FROM TREATED FLORISIL TUBES

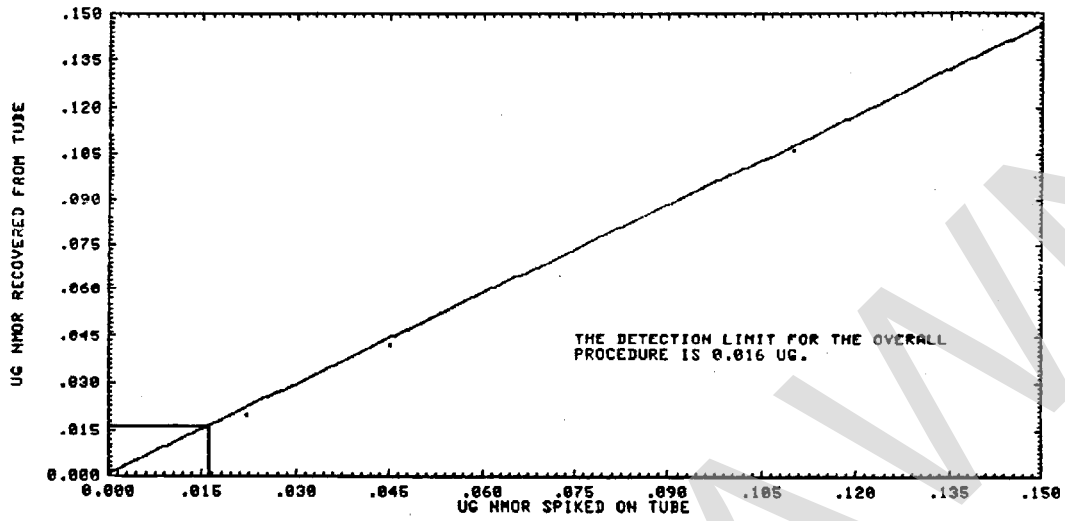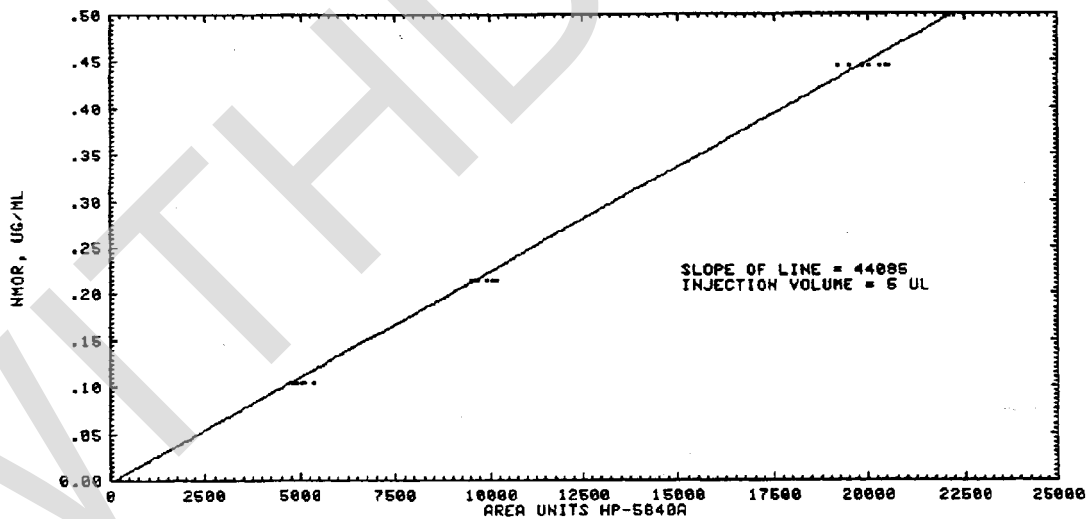Figure 4.2.1. Detection limit of the overall procedure for N-nitrosomorpholine desorbed from treated Florisil tubes.

Note: OSHA no longer uses or supports this method (December 2019).

Figure 4.2.2. Detection limit of the overall procedure for N-nitrosomorpholine desorbed from treated Polar Partition tubes.

Figure 4.4.1. N-nitrosomorpholine calibration curve.

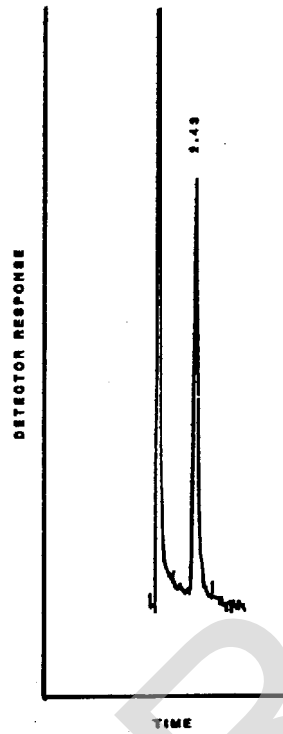Note: OSHA no longer uses or supports this method (December 2019).

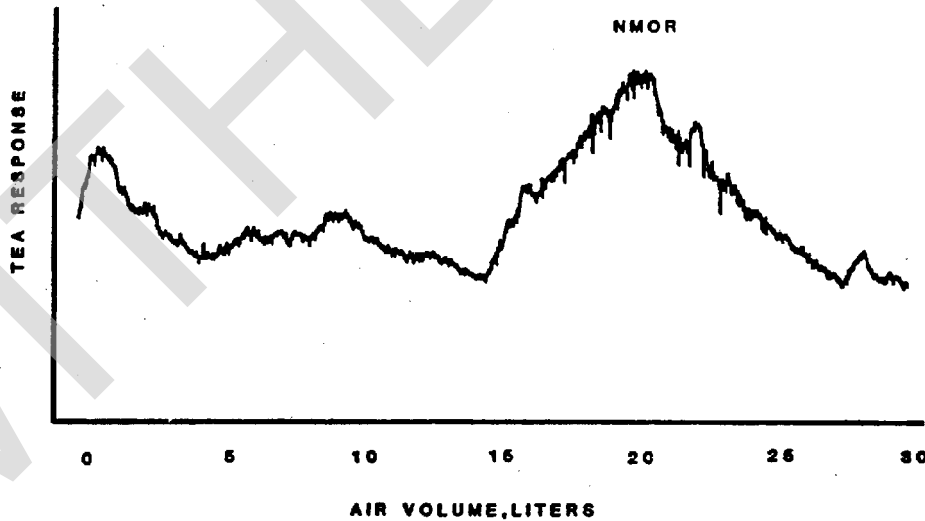Figure 4.4.2. A representative N-nitrosomorpholine chromatogram at the target concentration.

Figure 4.6.1. The rate at which N-nitrosomorpholine is desorbed from 50 mg of Polar Partition adsorbent at ambient relative humidity and temperature.

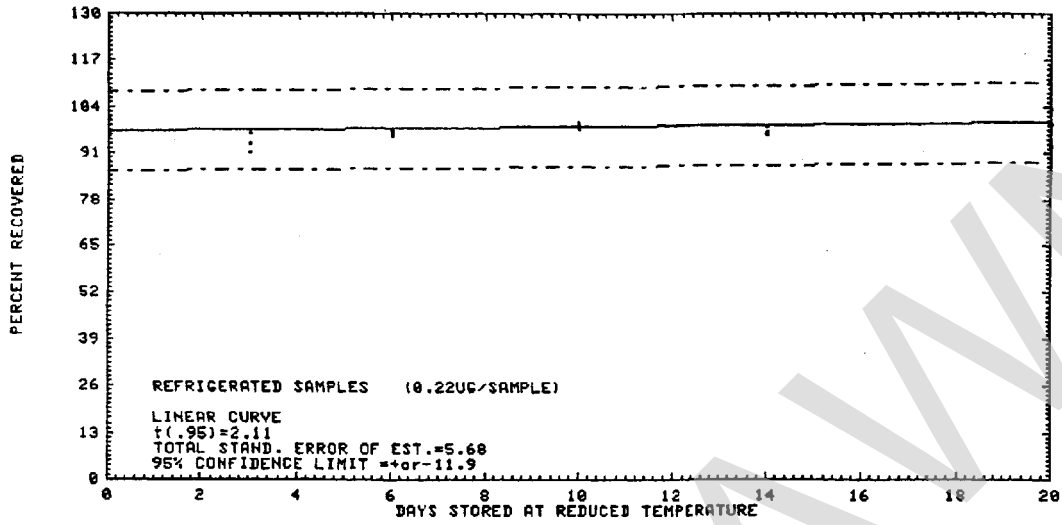Note: OSHA no longer uses or supports this method (December 2019).

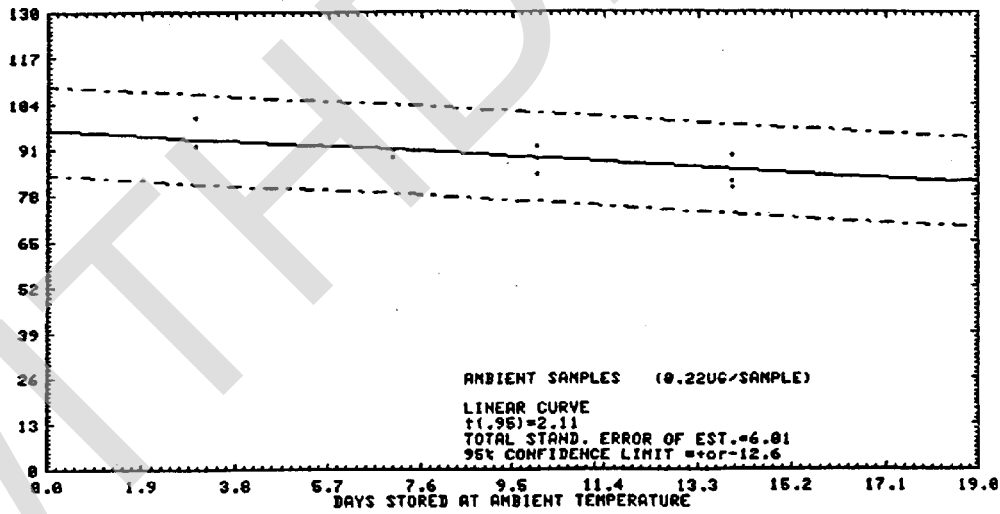Figure 4.6.2. Refrigerated temperature storage test for N-nitrosomorpholine.

Figure 4.6.3. Ambient temperature storage test for N-nitrosomorpholine.

Note: OSHA no longer uses or supports this method (December 2019).

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