Designation: F 1313 – 90 (Reapproved 1999)

Standard Specification for Volatile *N*-Nitrosamine Levels in Rubber Nipples on Pacifiers[1]

This standard is issued under the fixed designation F 1313; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This specification applies to the nitrosamine content of rubber used in the manufacture of nipples for infant pacifiers.

1.2 This specification does not apply to plastic nipples (on pacifiers).

1.3 The purpose of this specification is to establish a maximum level of allowed nitrosamines in rubber nipples and to outline a uniform testing method to determine such level.

1.4 The values stated in SI units are to be regarded as standard. The values given in parentheses are for information only.

1.5 The following precautionary statement pertains only to the test method portions, Sections 5, and Appendix X4 of this specification. *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazards are given in Appendix X2.

2. Terminology

2.1 *Definitions:*

2.1.1 *lot*—a normal production run or, in the case of imports, a shipment of items produced in the same time frame.

2.1.2 *nitrosamines*—chemically active compounds principally formed by the reaction of amines with oxides of nitrogen present in the environment.

3. Significance and Use

3.1 This specification is intended for use in reducing the normal exposure to nitrosamines.

3.2 This specification refers only by way of example to the eight volatile *N*-nitrosamines identified below:

3.2.1 *N*-nitrosodimethylamine,
3.2.2 *N*-nitrosodiethylamine,
3.2.3 *N*-nitrosodibutylamine,
3.2.4 *N*-nitrosomorpholine,
3.2.5 *N*-nitrosopiperidine,
3.2.6 *N*-nitrosopyrrolidine,
3.2.7 *N*-ethylphenylnitrosamine.

4. Test Method

4.1 Determine nitrosamine levels by using either the methylene chloride extraction method described in the collaborative study conducted by the National Center for Toxicological Research[2] or the Food and Drug Administration method.[2]

5. Acceptable Level

5.1 A test sample of nipples, drawn from a standard production lot, shall not contain more than 10 ppb (in each of 3 aliquots) of any one nitrosamine. In addition, the total nitrosamines of the sample shall not exceed 20 ppb.

5.2 Each manufacturer or distributor of the product shall test the product in such a manner and at such intervals to ensure compliance in accordance with the methodology prescribed by the test procedure utilized. Records of all testing shall be retained for a period of up to three years.

6. Report

6.1 Report the following information:

6.1.1 Lot number,
6.1.2 Date samples,
6.1.3 Date tested,
6.1.4 Individual nitrosamine content, and
6.1.5 Total nitrosamine content.

[1] This specification is under the jurisdiction of ASTM Committee F15 on Consumer Products and is the direct responsibility of Subcommittee F15.22 on Toy Safety.

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[2] Available from the Superintendent of Documents, U.S. Government Printing Office, North Capitol and H Streets, NW, Washington, DC 20401.

APPENDIXES

(Nonmandatory Information)

X1. BACKGROUND

X1.1 This specification provides the rationale for the drafting of a voluntary product standard establishing acceptable levels and testing procedures for nitrosamines contained in children's rubber pacifiers.

X1.2 Some nitrosamines are known to be potent animal carcinogens and are suspected human carcinogens. In 1981, the West German Government enacted regulations limiting the amount of preformed nitrosamine in rubber pacifiers. Nitrosamines are formed from amines used as accelerators during vulcanization of the rubber or are unintentional trace substances present in stabilizers used in the manufacturing process.

X1.3 In 1982, the Consumer Product Safety Commission (CPSC) began meeting with rubber pacifier manufacturers and importers (most are imported), drawing their attention to both the carcinogenic potential as measured by laboratory bioassays on rodents and the results of an audit of those pacifiers on the market. The audit revealed nitrosamine levels ranging from "non-detectable" to as much as hundreds of parts per billion (ppb). The Toy Manufacturers of America (TMA) undertook to coordinate a program to lower the levels of nitrosamines and validate a single test method that could be duplicated in laboratories worldwide. This effort was a joint, round-robin program with the CPSC, the National Center for Toxicological Research (NCTR) and pacifier manufacturers/importers. Another method of testing has been detailed by the Food and Drug Administration in their program to reduce nitrosamine levels in nursing nipples.

X1.4 This specification currently recognizes two test methods, one developed by the National Center for Toxicological Research (NCTR) (see Appendix X3), and one which is known as the Food and Drug Administration (FDA) method (see Appendix X4). Both methods have been corroborated and adopted as an approved method by the Association of Official Analytical Chemists. The process by which these methods were corroborated and adopted ensures that the methods are reproducible both within and between laboratories and that the methods provide equivalent test results. Several government and independent laboratories participated in the corroborative study in which coded quadruplicate samples of three composites were sent to each laboratory for analysis and tally, conclusively providing evidence of reproducibility among laboratories.

X1.5 The Consumer Product Safety Commission uses the NCTR method in analyzing pacifiers for nitrosamine content under its enforcement policy.[3] The FDA utilizes the FDA method in its Compliance Policy Guide, 7117.15.[4] The CPSC and NCTR staffs characterize the NCTR method as cheaper, faster, and more reproducible, although both the NCTR and FDA have affirmed that their two methods give essentially the same results in their laboratories.

X1.6 The test methodologies contained in Appendix X3 and Appendix X4 define sample sizes and contain the requisite and prescribed procedure for sampling from a lot to be tested.

X1.7 On December 27, 1983, the CPSC issued a statement of policy that rubber pacifiers are hazardous substances as defined in Section 2(g) of the Federal Hazardous Substances Act and are banned if they contain more than 60 ppb of nitrosamines as measured by the NCTR methylene chloride extraction test, effective January 1, 1984.

X1.8 A collaborative study between the NCTR, manufacturers/importers and leading testing laboratories was initiated to validate the test for consistent results between laboratories. Manufacturers and importers have continued to work with manufacturing processes and independent laboratories to reduce nitrosamine levels during this period. Significant progress has been made since the start of the program.

X1.9 In June, 1985, a group of manufacturers met with the Toy Manufacturers of America to draft a voluntary specification. That specification was presented to a task force of consumers and manufacturers on August 14, 1985 at ASTM Headquarters. This specification is the result of the corrections and suggestions made at that meeting, as well as comments from formal ASTM balloting procedures.

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[3] Federal Register 48, No. 249, pp. 56988–56990, available from Superintendent of Documents, U.S. Government Printing Office, North Capitol and H Streets, NW, Washington, DC 20401.

[4] Federal Register 49, No. 252, pp. 50789–50790, available from Superintendent of Documents, U.S. Government Printing Office, North Capitol and H Streets, NW, Washington, DC 20401.

X2. HAZARD ANALYSIS

X2.1 The scientific community in Europe, Canada and the United States has concluded that nitrosamines are suspected human carcinogens. However, the actual risk to infants who use rubber pacifiers is probably very small. In fact, a risk assessment study conducted by the Rubber Manufacturers Association involving infant feeding nipples concluded on a worst case basis that the lifetime risk to a user of infant nipples (having 60 ppb nitrosamines) was one in 23 million. However, the Toy Manufacturers Association has approached this problem, accepting that high levels of nitrosamines are unacceptable and that low levels of 20 ppb, that generally represent unavoidable contamination, are achievable.

X3. PROCEDURE FOR ANALYSIS OF *N* -NITROSAMINES IN PACIFIERS—A COLLABORATIVE STUDY

X3.1 *Reagents, Apparatus, and Pacifiers*—All solvents were distilled in glass[5] and all other reagents were chemically pure grade.

X3.1.1 *N-Nitrosamine Standard Stock*:

X3.1.1.1 *External Standard Stock*—Ten µg/mL in ethanol of 7 *N*-nitrosamine mixture.[6]

X3.1.1.2 *Internal Standard Stock*—A solution of NDPA (5 µg/mL in ethanol).

X3.1.2 *Pacifiers.*[7]

X3.1.3 *Mineral Oil*— White, light weight Saybolt viscosity 125/135.[8]

X3.1.4 *Nitrosation Inhibitor*—Ten mg alpha-Tocopherol/mL mineral oil.

X3.1.5 *Keeper Solution:*

X3.1.5.1 *For K-D Evaporation*—Eighty mg mineral oil/mL dichloromethane.

X3.1.5.2 *For N_2 Blowdown*—Twenty mg mineral oil/mL iso-octane.

X3.1.6 *ThermoSorb/N*[7] *Cartridges*—Used as received for quantitative trapping of volatile *N*-nitrosamines.

X3.1.7 *Variable Temperature Oil Bath*—Thermostatically controlled oil bath capable of operating at 150 ± 3°C and of moving vertically with aid of a lab jack.[9]

X3.1.8 *Purge and Trap Apparatus*—The apparatus shown in Fig. X3.1 contains the following parts:

X3.1.8.1 Argon (Ar) gas cylinder and gage;[10]

X3.1.8.2 Metering valve;

X3.1.8.3 Purge gas manifold 4-position;

X3.1.8.4 Nalgene[11] needle valve Type CPE (No. 6400-0125);

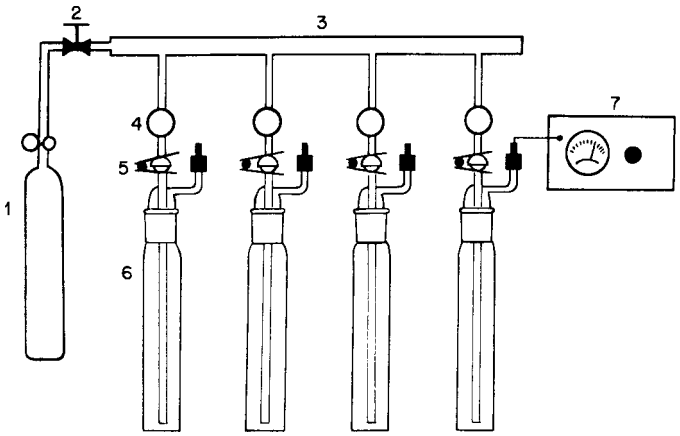

FIG. X3.1 Diagram of Purge and Trap Apparatus Equipped With Four Impinger Tubes

X3.1.8.5 Ground glass outer joints with pinch clamps, 18/7;[12]

X3.1.8.6 Impingers, 50 mL graduated glass tubes with 24/40 clear-seal grease free joints, 18/7 ground glass ball joints, and 1 mm inside diameter nozzle approximately 5 mm above the bottom of the impinger;[13] and

X3.1.8.7 *Variable Scale Flow-Check*[14]—Calibrated for purge rate in mL/min, of argon. A bubble meter for measuring gas flow rates for a gas chromatograph may be substituted.

NOTE X3.1—Do not use any rubber tubing, gaskets, o-rings, or any other items made of rubber in any part of this method.

X3.2 *Description and Use of the Purge and Trap Apparatus*—The apparatus shown in Fig. X3.1 was designed for the high temperature purging and trapping of seven volatile nitrosamines from a concentrated sample extract/mineral oil mixture on four samples simultaneously. A cylinder containing prepurified argon (Ar) gas equipped with a high pressure regulator was used to supply 20 psig to a flow metering valve that regulates the final purge flow through the samples. The gas stream was diverted into a tubular stainless steel manifold 250

[5] Available from Burdick and Jackson, Muskegon, MI, or equivalent, has been found satisfactory for this purpose.

[6] Available from Thermo Electron Corp., Waltham, MA, or equivalent, has been found satisfactory for this purpose.

[7] Available from Consumer Products Safety Commission, Bethesda, MD, or equivalent, have been found satisfactory for this purpose.

[8] Number 6358, available from Mallinckrodt, Paris, KY, or equivalent, has been found satisfactory for this purpose.

[9] Available from The Lab Apparatus Co., Cleveland, OH, or equivalent, has been found satisfactory for this purpose.

[10] Available from Air Products Specialty Gas, Tamaqua, PA, or equivalent, has been found suitable for this purpose.

[11] Available from Nalge Co., Rochester, NY, or equivalent has been found suitable for this purpose.

[12] Number 772398, available from Wheaton Scientific, Millville, NJ, or equivalent, has been found suitable for this purpose.

[13] Number 753463, available from Wheaton Scientific, Millville, NJ, or equivalent, has been found suitable for this purpose.

[14] Number 7083, available from Alltech Associates, Houston, TX, or equivalent, has been found suitable for this purpose.

by 20 mm outside diameter containing four exit tubes spaced 50 mm apart and measuring 40 by 10 mm outside diameter. Each of these tubes were coupled using 9.52 mm (⅜ in.) Tygon tubing to Nalgene[11] needle valves that serve dual purposes: as a shut off valve when assaying less than four samples; and for making minor adjustments in purge rate due to slight differences in flow characteristics of the impinger and ThermoSorb/N[6] cartridges. An 18/7 ground glass outer spherical joint was attached to the Nalgene[11] valve to permit a quick, gas tight connection to the 18/7 ground glass ball joint on the impinger inlet using the appropriate pinch clamp. As shown in Fig. X3.2 the impingers were assembled by inserting the glass nozzle (1 mm inside diameter orifice) into the sample mixture and coupling the 24/40 grease free male and female joints together forming a leak free seal. Once sealed, the Ar gas was allowed to purge through the sample mixture, through the outlet tube of the impinger (see Fig. X3.2). Tygon tubing was used to connect the impinger outlet tube to the inlet side (marked "AIR IN") of the ThermoSorb/N[6] cartridge, that is simply a standard male luer syringe connector. The purged volatile *N*-nitrosamines were then collected on the sorbent contained in the cartridge with Ar effluent exiting from the female luer connector. The flow rate of Ar was measured directly from the cartridge with a variable scale flow meter that had beenpreviously calibrated for flow rate of Ar gas (mL/min). A bubble meter can be substituted for the variable scale flow meter. The temperature of the sample mixture during purge was controlled by immersing the impinger up to the sample volume mark (approx. the 25 mL line) in a thermostatically controlled oil bath capable of operation isothermally up to 150°C. The gas manifold, as well as each of the impingers, were secured by clamps to a support grid; therefore, the oil bath was moved vertically in and out of position for high temperature purge.

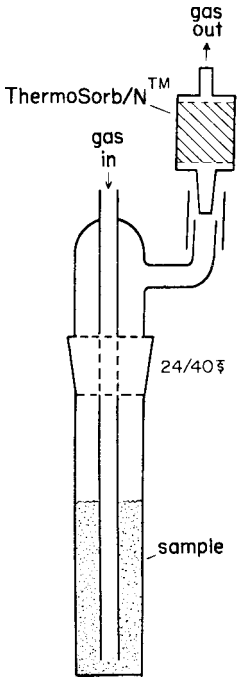

FIG. X3.2 Diagram of Close-Up of Impinger Tube Fitted With a ThermoSorb/N[7] Cartridge

X3.3 *Procedure for Extraction and Clean-Up of Pacifier Samples:*

X3.3.1 Prepare a composite of pacifier rubber by cutting a sufficient number of individual nipples for your replicate requirements from a single lot into 1 to 2 mm chips using stainless steel scissors and tweezers. Homogenize the composite by freezing in a stainless steel blender jar with liquid nitrogen, decanting the liquid N_2, blending at high speed for 1 to 2 min. Immediately transfer the homogenized composite to a glass jar with an aluminum foil lined lid and allow to equilibrate to ambient temperature.

X3.3.2 Accurately weigh 5 g samples from the composite into a 250-mL round bottom flask and add 100 mL dichloromethane.

X3.3.3 Spike the contents of the flask with 2 mL of the internal standard (50 ng/mL NDPA). Seal the flask and soak the contents overnight (16 to 21 h) at ambient temperature.

X3.3.4 Then transfer the extract and rubber pieces to a glass extraction thimble fitted with a coarse porosity glass frit in a Soxhlet extraction apparatus.

X3.3.5 Rinse the 250 mL round bottom flask with 25 mL dichloromethane, that was also transferred to the Soxhlet apparatus.

X3.3.6 Extract the rubber pieces for 1 h in the apparatus at the rate of eight cycles per hour.

X3.3.7 After cooling, transfer the dichloromethane extract to a 250-mL Kuderna Danish (K-D) evaporator.

X3.3.8 Then rinse the Soxhlet extraction flask with two 10-mL portions of dichloromethane and combine with the 125-mL extract.

X3.3.9 Add 1 mm of keeper solution and a few boiling chips[15] to the extract.

X3.3.10 Evaporate the extract in the K-D unit using a 3-ball Snyder column on a 55°C water bath until the volume is reduced to 3 to 4 mL.

X3.3.11 Cool the K-D unit to room temperature allowing excess solvent in the Snyder column to rinse down the walls of the unit into the 4-mL K-D tube (totaling 3 to 4 mL).

X3.3.12 After removing the 250-mL reservoir and the 3-ball Snyder column, reduce the volume of the extract to 2 mL in the same K-D tube under a gentle stream of nitrogen (about 50 mL/min) and transfer the 2 mL extract using a disposable Pasteur pipet with two 1-mL mineral oil rinses to a 50-mL purge and trap apparatus containing 20 mL of mineral oil and 1 mL of 10-mg/mL alpha-tocopherol in mineral oil as a nitrosation inhibitor.

X3.3.13 Assemble the purge and trap apparatus with ThermoSorb/N[6] cartridges connected to exit tubes with a Tygon connector.

X3.3.14 Adjust the argon flow rate to 400 mL/min through the ThermoSorb/N[6] cartridge within ±5 % (that is 380 to 420 mL/min Ar).

NOTE X3.2—The flow rate should be checked intermittently during purging, especially within the first 15 min because of the initial increase in temperature of the sample.

[15] "Boileezers", a product of Fisher Scientific, Memphis, TN, or equivalent, have been found suitable for this purpose.

X3.3.15 Then immerse the purge tubes up to the sample line, or about the 25-mL mark in a 150 ± 3°C oil bath for 1.5 h.

X3.3.16 Remove and tightly cap the cartridge.

NOTE X3.3—This step is a good stopping point because the cartridge can be eluted the following day if time is a factor.

X3.3.17 Elute the cartridge using a 10 or 20-mL glass Luer-lok syringe connected to the female Luer-lok adapter (air exit side) with 20 mL of acetone: dichloromethane (1:1; v/v), that was collected in a 30-mL culture tube.

NOTE X3.4—The 30-mL tube(s) should be scored with a file or a piece of tape placed at the 5-mL volume mark.

X3.3.18 Evaporate the extract to approximately 5 mL and then transfer with three 1-mL rinses of dichloromethane to a 10-mL graduated tube.

NOTE X3.5—For NDBA, evaporate the sample to 1 mL for detection levels less than 10 ppb.

X3.3.19 After addition of 0.5 mL of keeper solution (see X3.1.1.2), evaporate the sample (volume = 8.5 mL) to 2 mL under a gentle stream of nitrogen.

NOTE X3.6—If the 2 mL sample cannot be analyzed the same day as evaporated, then it would be advantageous to refrigerate the sample at a larger volume (that is 4 to 5 mL) and evaporate the next day prior to analysis by gas chromatography-thermal energy analysis (GC-TEA).

X3.3.20 The 2-mL sample was analyzed by injecting an 8 µL aliquot into the GC-TEA.

X3.4 *Gas Chromatography-Thermal Energy Analysis (GC-TEA)*—The gas chromatograph (GC) used was a Hewlett-Packard Model 5710A instrument[16] equipped with a 6-ft glass column (4 mm inside diameter) packed with 10 % Carbowax 20M/2 % KOH on 80/100 mesh Chromosorb W AW.[17] The glass column conditioned at 215°C overnight prior to use, was operated in the temperature program mode from 150 to 190°C at 4°C/min. The injection port temperature was 250°C. The carrier gas was prepurified Ar gas that flowed at a rate of 40 mL/min. The GC column was interfaced to a thermal energy analyzer Model 502[6] via an 3.17 mm (⅛ in.) outside diameter stainless steel tube connected by Swagelok fittings and operated at 170°C. The TEA pyrolysis chamber was kept at 500°C in the GC mode. The oxygen flow to the ozonator was 10 mL/min. The cold trap was kept at −150°C using a liquid nitrogen-2 methylbutane slush bath. The pressure of the reaction chamber was approximately 0.9 torr. The TEA detector response was recorded on a Hewlett Packard 3380A[17] integrator. All sample injections into the GC-TEA system were 8 µL aliquots of the sample extracts.

X3.5 *Quantitation*—Quantitation is based on the internal standard technique.

X3.5.1 Dilute the external standard stock solution with dichloromethane to 50, 100, and 200 ng/mL to be used as working standards for analysis. Inject 8 µL into the GC-TEA to determine responses (peak heights) of NDPA and the other nitrosamines for use in the internal standardization calculation.

X3.5.2 Inject 8 µL of each 2-mL unknown sample extract into the GC-TEA. Determine responses (peak heights) of NDPA and any other nitrosamines detected for use in the internal standardization calculation.

X3.5.3 The calculation of results is as follows:

$$\text{amount of } y \text{ (ppb)} = \frac{\text{peak height}_{y(a)} \times \frac{\text{ng}_y}{\text{peak height}_{y(b)}}}{\text{peak height}_{\text{NDPA }(a)} \times \frac{\text{ng}_{\text{NDPA}}}{\text{peak height}_{\text{ NDPA }(b)}}} \times \frac{100 \text{ ng}}{\text{sample weight } (g)} \quad \text{(X3.1)}$$

where:

amount of y = ppb of nitrosamine y in sample,

$\text{peak height}_{y(a)}$ = peak height in mm of nitrosamine y in sample,

$\text{peak height}_{\text{NDPA }(a)}$ = peak height in mm of NDPA (internal standard) in sample,

$\frac{\text{ng}_y}{\text{peak height}_{y\,(b)}}$ = ng of nitrosamine y per millilitre in the external standard divided by the peak height in millimetres of nitrosamine y in the external standard,

$\frac{\text{ng}_{\text{NDPA}}}{\text{peak height}_{\text{NDPA }(b)}}$ = ng of NDPA per millilitre in the external standard divided by peak height in millimetre of NDPA in the external standard,

sample weight *(g)* = grams of rubber sample analyzed, and

100 ng = total ng of NDPA (internal standard) added to the sample.

X3.6 *Sample Homogenization Procedure*—From each pacifier lot, remove eight to 24 units for analysis depending upon the number of pacifier nipples (0.5 to 1.6 g/nipple) needed to analyze duplicate 5 g rubber samples. Excise the nipples, using dichloromethane rinsed stainless steel forceps and scissors, from the plastic or rubber base and cut into 1 to 2-mm chips. Many of the samples exhibit a stickiness after being cut, making homogenization very difficult. In order to break up the large clumps of rubber, transfer the sample into a 70 by 155-mm stainless steel Sorvall omni-mixer cup. Pour liquid nitrogen into the cup to cover up all of the rubber chips. Then discard the excess liquid nitrogen into a waste Dewar flask using insulated gloves to handle the extremely cold metal cup. Homogenize the frozen rubber chips by attaching the cup to the mixer housing and setting the speed to approximately 40 % of the maximum for 1 min. Remove the cup containing the homogenized rubber chips from the mixer. Pour the chips

[16] A product of Hewlett-Packard, Avondale, PA.

[17] Number 1-1805, available from Supelco, Inc., Bellefonte, PA, or equivalent, has been found suitable for this purpose.

into a 100-mL volume tinted glass sample jar with an aluminum foil lined screw cap. Then store composited sample in a freezer at −20°C until needed for extraction.

NOTE X3.7—Be careful to avoid addition of any small balls of powdered rubber that might be formed in the blending process.

X4. METHOD

X4.1 *Reagents:*

X4.1.1 *Dichloromethane*, distilled in glass.

X4.1.2 *Sodium Hydroxide*, reagent grade.

X4.1.3 *Barium Hydroxide*, reagent grade.

X4.1.4 *Water*, doubly distilled.

X4.1.5 *Sodium Carbonate*, reagent grade, anhydrous, granular.

X4.1.6 *Sodium Sulfate*, reagent grade, anhydrous, granular.

X4.1.7 *Carborundum Grains*[18]

X4.1.8 *N-nitrosamine Standard Solutions*—Prepare for each suspected *N*-nitrosamine a stock solution (A) 1000 mg/L DCM. Dilute each solution, 10 to 100 mL with DCM (100 µg/mL) (B). Prepare a combined solution (C) to contain 0.5 µg/mL DCM of each nitrosamine by pipeting 1.0 mL ofeach solution B into a volumetric flask and diluting to 200 mL with DCM. Prepare internal standard solution, 100 ng/mL (D) by diluting 10 mL stock solution of *NDPA*, B, to 100 mL and further diluting 1 to 100 mL with DCM (standard combined solution) (C) may be prepared from commercially available diluted standards).

X4.2 *Apparatus*—Usual laboratory equipment and glassware and also the following:

X4.2.1 *Soxhlet Extractor*:

X4.2.1.1 *Extraction Tube*, 40 mm with standard taper 45/50 condenser connection and standard taper 24/40 flask connection.

X4.2.1.2 *Condenser*, Allihn type, standard taper 45/50 joint.

X4.2.1.3 *Flask*, 250 mL capacity, standard taper 24/40 joint.

X4.2.1.4 *Extraction Thimble*, 80 by 33 mm, 30 mL capacity, coarse porosity.

X4.2.2 *Evaporative Concentrator*, Kuderna Danish (KD), 250 mL capacity with standard taper 24/40 column connection and standard taper 19/22 lower joint.

X4.2.2.1 *Concentrator Tube*, size 425, standard taper 19/22 joint, 4 mL capacity.

X4.2.2.2 *Distilling Column*, Synder, standard taper 24/40 joint, three sections.

X4.2.3 *Filtering Funnel*—Coarse porosity, 60 mL capacity.

X4.2.4 *Gas Chromatograph*—Hewlett Packard 5710A,[19] or equivalent.

X4.2.5 *Thermal Energy Analyzer*—(TEA) Model 502L.[6]

X4.3 *Procedure:*

X4.3.1 *Blank Test*— To ensure absence of interfering peaks, check DCM separately and all reagents prior to use by performing a total reagent blank. Check DCM by concentrating 200 to 0.5 mL in KD apparatus and injecting 8 µL into GC-TEA. Check water by partitioning 100 mL water and three 50 mL portions of DCM. Dry, and concentrate DCM to 0.5 mL in KD apparatus and inject 8 µL into GC-TEA. If interferences occur, discard.

X4.3.2 *Sample Preparation*—Samples to be provided by associate referee.

X4.3.3 *Extraction*— Place cut-up sample (5 g) in a 250-mL glass stoppered round bottom flask. Add 100 mL DCM to flask, stopper and hold overnight (12 to 18 h). Attach a 250-mL flask containing boiling chips to Soxhlet extractor. Quantitatively transfer DCM extract and nipple pieces to extraction thimble held in Soxhlet extractor, using a funnel. Wash flask twice with 12 mL DCM and add to the extractor. Spike Soxhlet with 1.0 mL NDPA solution (D). Attach Soxhlet to water cooled condenser. Attach heating mantle to flask and extract for 1 h at a Variac setting of (102V). Remove heating mantle and allow extractor to cool 15 min. Siphon any DCM remaining in the Soxhlet into the flask.

X4.3.4 *Distillation*— To DCM extract add boiling chips, 100 mL 5 *N* sodium hydroxide and 2 g barium hydroxide. Attach to atmospheric distillation apparatus. Carefully distill DCM at Variac setting of 30 % (36V). Discard DCM distillate. Adjust Variac to 71 % (86V) and collect 70 mL aqueous distillate in a calibrated 250-mL separatory funnel.

X4.3.5 *Liquid-Liquid Partition*—Add 300 mg anhydrous sodium carbonate to the distillate. Add 50 mL DCM and shake vigorously for 1 min. Separate organic and aqueous layers. Repeat DCM extraction twice. Combine DCM extract in a 250 mL separatory funnel. Pass DCM extracts through 30 g anhydrous sodium sulfate (held in a 60-mL course sintered-glass filtering funnel and pre-washed with 25 mL DCM) into a 250-mL KD evaporator. Wash sodium sulfate with 15 mL DCM and add to the KD.

X4.3.6 *Concentration of Extract*—Add 2 or 3 carborundum grains, attach three-section Snyder column and carefully concentrate DCM extract, at a rate of 1 mL/min, to 4 mL in a 60°C water bath. Remove KD from water bath and allow to cool 15 min. Remove concentrator tube and carefully concentrate to 1.0 mL under a gentle stream of nitrogen. Stopper and hold for GC/TEA analysis.

NOTE X4.1—Concentration of oily samples can be facilitated by immersing tip of concentrator tube in beaker containing warm water −40°C.

X4.3.7 *GC-TEA Analysis*— GC/TEA conditions are as follows:

Column	Glass packed with 10 % Carbowax 1540 and 5 % potassium hydroxide on 100/120 Chromosorb WHP. Length is 2.7 m and inside diameter of 4 mm.
Carrier gas	Argon, 40 mL/min (or equivalent).
Column temperature	Programmed 100 to 180°C at 4°C/min.

[18] Available from Carborundum Corp., Niagara Falls, NY, or equivalent, has been found suitable for this purpose.

[19] Available from Hewlett-Packard, Avondale, PA.

Injection port	200°C
TEA furnace	450°C
Attenuation	*N*-nitrosamine standard, X8 to X16. Sample extracts, depending on *N*-nitrosamine level.
Trap	Liquid nitrogen or CTR gas stream filters.[6]
	Inject 8 µL *N*-nitrosamine standard solution (C) and NDPA solution (D) and carry out chromatographic analysis. Using the same conditions, inject 8 µL concentrated sample extract and carry out chromatographic analysis. Measure peak response of *N*-nitrosamines in standard and sample extracts that occur at the same retention time.

X4.4 *Method of Calculation:*

V = volume of sample extract (=1.0 mL),

C_s = concentration of standard (=0.5 µg/mL),
P_{sp} = peak response of sample,
P_{st} = peak response of standard,
S_{wt} = sample weight, and
N = *N*-nitrosamine concentration (µg/Kg) in sample.

thus:

$$N = \frac{Cs\ V\ Psp1000}{P_{st}\ S_{wt}} \text{ and} \quad \text{(X4.1)}$$

$$\%\ \text{recovery}\ \ NDPA = \frac{P_{sp} \times 100.}{P_{st}} \quad \text{(X4.2)}$$

X4.4.1 Report results to the nearest 0.1 µg/Kg (ppb).