Federation of the Employment Accidents Insurance Institutions of Germany
(Hauptverband der Berufsgenossenschaften)
Centre for Accident Prevention and Occupational Medicine
Alte Heerstraße 111, 53757 Sankt Augustin
Expert Committee Chemistry

Carcinogenic substances Order number: BGI 505-62E
Established methods Issue: December 1996

Method for the determination of *N*-nitrosomethylphenylamine (NMPA) and *N*-nitrosoethylphenylamine (NEPA)

Method tested and recommended by the Berufsgenossenschaften for the determination of *N*-nitrosomethylphenylamine (NMPA) and *N*-nitrosoethylphenylamine (NEPA) in working areas after discontinuous sampling.
For the assessment of working areas, only stationary sampling is possible:

1 Sampling with a pump and adsorption of gaseous nitrosamines in an annular denuder, gas chromatography after extraction and concentration of the extract.
"NMPA-NEPA-1-GC"
(Issue: December 1996)

IUPAC name:	**CAS No:**
N-nitrosomethylphenylamine	614-00-6
N-nitrosoethylphenylamine	612-64-6

1 Sampling with a pump and adsorption in an annular denuder, gas chromatography after extraction and concentration of the extract

This method permits the determination of *N*-nitrosomethylphenylamine (NMPA) and *N*-nitrosoethylphenylamine (NEPA) concentrations in working areas averaged over the sampling time after stationary sampling. A diffusion-based gas phase extractor (denuder) is used for sampling NMPA and NEPA. The named nitrosamines are selectively trapped. The secondary amines (methylphenylamine and ethylphenylamine) always present in the work areas in question pass through the sampling system without being trapped.

Principle: With a pump a measured air volume is drawn through the denuder at a flow rate of 8 L/min for 30 minutes.
Gaseous NMPA and NEPA are absorbed on the specially coated inside walls of the denuder. The absorbed nitrosamines are desorbed together with the coating (sink) with a mixture of 0.05 M sodium hydroxide solution and a toluene/dichloromethane mixture. Clean-up is carried out by liquid/liquid extraction. After further concentration of the sample the absorbed nitrosamines are analysed using a gas chromatograph equipped with a thermal energy analyser (TEA) detector.

Technical data:

Quantification limit: absolute: 0.1 ng NMPA or NEPA,
relative: 0.5 µg/m^3 NMPA or NEPA for 240 L air sample, 250 µL sample solution and 2 µL injection volume.

Selectivity: In the sampling system the named nitrosamines are separated from the corresponding amines, which are not collected. Semi volatile nitrosamines (e. g. N-nitrosomorpholine, N-nitrosopyrrolidine or N-nitrosodibutylamine) are incompletely deposited in the denuder system under the conditions described here. The TEA detector system is selective in combination with gas chromatographic separation. Because of the selectivity of the sampling system, interference from other organic nitrogen compounds is not to be expected.

Advantages: Selective and artefact-free determination from the gaseous phase.

Disadvantages: The sampling time is limited. Increased relative humidity (relative humidity >50%) leads to analytical values which are too low. Personal sampling is not possible.

Apparatus: Pump,
gas meter or flow meter,

annular denuder,
shaking machine,
centrifuge,
apparatus for concentrating solutions with an inert gas,
gas chromatograph with chemiluminescence detector (TEA detector).

Detailed description of the method

Contents

1 Equipment, chemicals and solutions

1.1 Equipment

For sampling:

Pump, suitable for a flow rate of 8 L/min (e.g. MF 70 from BW Meßtechnik GmbH, 52074 Aachen; GSA 50-1 from GSA Meßgerätebau GmbH, 41469 Neuss; gas sampler VR 03 from Desaga, 69168 Wiesloch; MCS-30 from SKC, supplier in Germany: MTC, 79379 Müllheim)

Gas meter or flow meter (e.g. rotameter, measuring range from 1–10 L/min, gas meter,

Soap bubble flow meter, e.g. Gilibrator from Gilian, supplier in Germany: GSM GmbH, Neuss-Nerf)

Denuder, four-fold annular denuder with plane ground joints and end caps coated with polytetrafluoroethylene (PTFE) and adapter for regulating the flow (supplier in Germany: Laborgroßhandlung G. Felser, 44229 Dortmund), preparation of the denuder, cf. Sect. 1.4; sketch, cf. Appendix, Sect. 8

For sample preparation and analysis:

3, 10, 100 and 1000 mL Volumetric flasks

3 and 5 mL Sample vials with PTFE-coated septa and crimp caps
15 mL Graduated centrifuge tubes
Graduated evaporating tubes with conical base for concentration to small volumes of 0.5–1 mL
1 mL Autosampler vials with PTFE-coated caps and appropriate 150 µL conical inserts
5, 10, 25, 100, 500 µL and 5 mL Syringes
5 mL Dispensette
Flat-bed shaker (e.g. IKA, 79217 Staufen)
Laboratory centrifuge
Apparatus for concentration of solutions with an inert gas (e.g. Restek-Amchro, 65812 Bad Soden)
Gas chromatograph with split/splitless injector and TEA detector (Thermedics, supplier in Germany: Isconlab, 69123 Heidelberg)
Evaluation unit

1.2 Chemicals

Toluene, analytical grade
Dichloromethane, analytical grade
Ethanol, analytical grade
Methanol, analytical grade
Sodium hydroxide
Sodium chloride
Triethanolamine
Tetraethylene glycol
N-Nitrosomethylphenylamine (NMPA)
N-Nitrosoethylphenylamine (NEPA)
N-Nitroso-*n*-butyl-*n*-propylamine (NBPA, internal standard)

Gases for operating the gas chromatograph and for sample preparation:
Helium, purity at least 99.999%
Oxygen, medical grade
Nitrogen, purity at least 99.996%
Synthetic air free of hydrocarbons

1.3 Solutions

NMPA stock solution:
Solution of 100 µg/mL NMPA in dichloromethane.
100 mL dichloromethane are added to 1 g of the nitrosamine, which is supplied in a special safety bottle (e.g. ISO-PACK, Sigma, 82041 Deisenhofen). 100 µL of the concentrated solution of NMPA is transferred to a 10 ml volumetric flask and diluted to the mark with dichloromethane.

NEPA stock solution:
Solution of 300 µg/mL NEPA in dichloromethane (e. g. Promochem, 46485 Wesel).

Stock solution for preparing dilutions:
Solution of 10 µg/mL NMPE and 10 µg/mL NEPA in dichloromethane.
300 µL NMPA stock solution and 100 µL NEPA stock solution are transferred to a 3 ml volumetric flask and diluted to the mark with dichloromethane.

NBPA stock solution 1:
Solution of 1.3 mg/mL NBPA in ethanol.
In a 100 mL volumetric flask 129.5 mg of the nitrosamine, which are supplied in a special safety bottle (e. g. ISO-PACK, Sigma, 82041 Deisenhofen) is diluted to the mark with ethanol.

NBPA stock solution 2:
Solution of 26 µg/mL NBPA in ethanol.
60 µL of NBPA stock solution 1 is transferred to a 3 ml volumetric flask and diluted to the mark with ethanol.

Calibration solutions:
Solutions of 0.02, 0.04, 0.08, 0.10, 0.20 and 0.50 µg/mL NMPA and NEPA with internal standard.
1 mL toluene is added to each of 6 sample vials. Then 2, 4, 8, 10, 20 and 50 µL toluene are removed and replaced by the same volumes of the stock solution for preparing dilutions. Moreover 1 µL NBPA stock solution 2 is added to each and shaken. With these solutions a concentration range of 0.04–1 µg/m^3 NMPA and NEPA is covered for a 240 L air sample and 250 µL sample solution.

Elution agent mixture 1:
In an 1 L volumetric flask approx. 2 g sodium hydroxide and 30 g sodium chloride are diluted to the mark with deionised water and shaken.

Elution agent mixture 2:
Mixture of dichloromethane/toluene with internal standard.
5 mL toluene is placed in a 100 mL volumetric flask. 5 µL NBPA stock solution 2 is added. The flask is filled to the mark with dichloromethane and shaken.

Coating solution:
In a 50 mL volumetric flask10 g triethanolamine and 10 g tetraethylene glycol are diluted to the mark with methanol and shaken. This solution can be kept for several weeks.

1.4 Preparation of the denuder

Before sampling, the annular denuder is rinsed with distilled water, then again with methanol and dried in a stream of air. For coating, the denuder is completely filled with the coating solution. Then the denuder is emptied and dried in a gentle stream of nitrogen (max. 7 L/min). Prepared in this way and closed with PTFE caps the denuder has a shelf life of 2–3 weeks.

2 Sampling

For sampling, the coated denuder is fitted vertically in the sampling system with the suction opening at the bottom. An adapter is placed before the denuder to ensure a laminar air flow. The way the system is constructed is sketched in Figure 1.

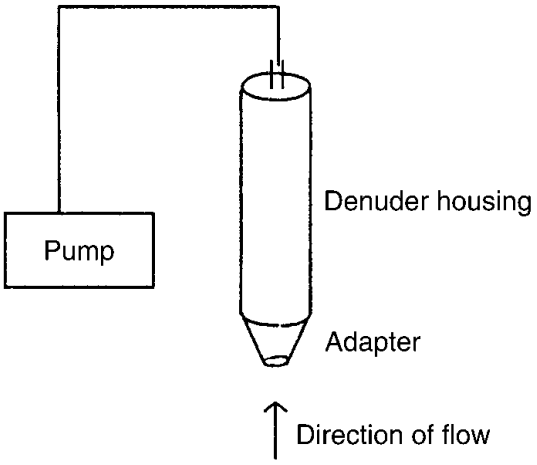


Fig. 1. Sketch of sampling system (cf. also Sect. 8, Appendix).

The flow rate is set to 8 L/min. The sampling period should not exceed 30 min. Sampling in this way for 30 minutes corresponds to an air sample volume of 240 L. After sampling, the denuder is closed with PTFE caps. The sample should be prepared and analysed immediately, if possible. After more than 7 days noticeable nitrosamine losses are observed.

3 Analytical determination

3.1 Sample preparation and analysis

5 mL of elution agent 1 and 5 mL of elution agent 2 are placed in the denuder. For an even wetting of the inner surface of the denuder and to mix the solutions well, the denuder is shaken on a flat-bed shaker for 20 min. The denuder must be placed on the shaker with its longitudinal axis in the direction of the shaking axis. To completely wet all the inner surfaces, the denuder must be rotated about the longitudinal axis after ten minutes.

The eluate is transferred to a graduated centrifuge tube and for better phase separation centrifuged for 20 min at 3000 rpm. Approximately 4 mL of the organic phase (bottom layer) is transferred with a 5 mL syringe to a graduated evaporation tube and in a gentle stream of nitrogen (rate of evaporation not greater than 1 mL/15 min) concentrated

to 150–300 µL (sample solution). Care must be taken that the sample is not warmed and that the nitrogen flow is so set that the surface of the sample is only slightly domed. The walls of the evaporation tube are rinsed with the sample solution and the concentrate transferred to an autosampler vial with conical insert. Analysis of the sample solution is carried out using GC/TEA.
To ensure that the solutions used do not contain any interfering substances, a coated, unloaded denuder is prepared and analysed (blank value).
To check the whole procedure a spiked denuder is prepared and analysed (control sample). The control analysis should yield a recovery of at least 70 %.
To spike the denuder, a short glass tube (evaporation tube) containing a defined volume of the stock solution for preparing dilutions (e. g. 5 µL) is attached to the front of the denuder. With a pump air is drawn through the denuder for 30 min at a flow rate of 8 L/min. For this purpose, the denuder should be placed horizontally as shown in Fig. 2.

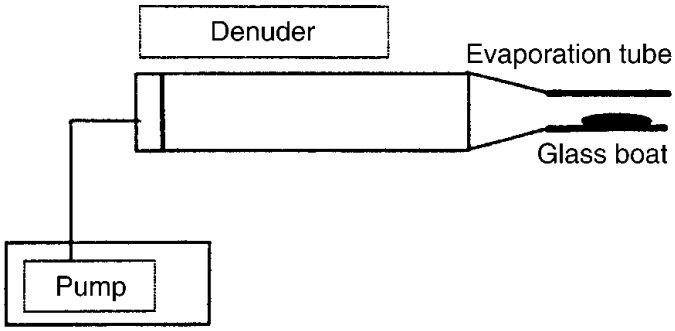


Fig. 2. Apparatus for spiking the denuder to test the preparation step.

3.2 Operating conditions for gas chromatography

The method was characterized under the following experimental conditions:

Apparatus:	Gas chromatograph Sichromat 1–4 (Siemens, Karlsruhe) equipped with TEA detector 543 (Thermedics Inc., supplier in Germany: Isconlab, 69123 Heidelberg), split/splitless injector and autosampler (Siemens, Karlsruhe)	
Pre-column:	Material:	Quartz capillary, not coated, deactivated (from Chrompack, Frankfurt)
	Length:	1 m
	Internal diameter:	0.53 mm
		The pre-column prevents early contamination of the GC column and when high loads are analysed should be regularly exchanged to protect the analytical column.

Column:	Material:	Quartz capillary
	Length:	60 m
	Internal diameter:	0.53 mm
	Stationary phase:	polyethylene glycol (CP-WAX 52 CB from Chrompack, Frankfurt)
	Film thickness:	1.0 µm
Temperatures:	Injector:	130 °C
	Furnace temperature programme:	
	Starting temperature:	90 °C, 2 min isothermal
	Heating rate:	6 °C/min
	Final temperature:	140 °C, 30 min isothermal
Detector:	Interface:	210 °C
	Pyrolysis furnace:	500 °C
Injection mode:	Splitless	
Split:	25 mL/min, after 3 min	
Carrier gas:	Helium, 6.2 mL/min	
Detector:	Oxygen for operating the ozone generator, 3.6 mL/min	
Molecular sieve filter:	CTR™-Gas-Stream-Filter (Thermo Electron Corporation, supplier in Germany: Isconlab, 69123 Heidelberg)	
Injection volume:	2 µL	

4 Evaluation

4.1 Calibration

Volumes of 2 µL of the calibration solutions are injected into the gas chromatograph with the autosampler. Quantification is carried out with the aid of NBPA as internal standard. The calibration function is linear in the range from 20 to 500 ng/mL.
The calibration factor f is calculated according to Equation (1) using the peak areas for NMPA and NEPA and the internal standard NBPA obtained from the calibration solutions:

$$f = \frac{F_{is} \cdot w_c}{F \cdot w_{is}} \quad (1)$$

Legend:
f Calibration factor for NMPA or NEPA
F_{is} Peak area for the internal standard
F Peak area for the nitrosamine
w_{is} Weight of the internal standard in 1 mL of the particular calibration solution in µg
w_c Weight of the nitrosamine in 1 mL of the particular calibration solution in µg

The calibration factor is more or less the same for all calibration solutions. The mean calibration factor $\bar{f}$ can be used for calculating the analytical result.

4.2 Calculation of the analytical result

The concentration of NMPA or NEPA in the air sample in µg/m^3 is calculated according to the Equations (2) and (3):
The weight of the nitrosamine is calculated according to the following equation:

$$w = \frac{F \cdot w_{\text{ise}} \cdot \bar{f}}{F_{\text{is}}} \tag{2}$$

$$c_{\text{w}} = \frac{w \cdot 1000}{V \cdot \eta} \tag{3}$$

Legend:
w Weight of the nitrosamine in the elution solution in µg
w_{ise} Weight of the internal standard in the sample solution (enriched elution solution) in µg
F Peak area for the nitrosamine
$\bar{f}$ Mean calibration factor
F_{is} Peak area for the internal standard
c_{w} Nitrosamine concentration in the sample air in µg/m^3
V Air sample volume in L
η Recovery rate

5 Reliability of the method

5.1 Accuracy and recovery

To determine the relative standard deviation of the method 2, 5 and 10 µL of the stock solution for preparing dilutions and 10 µL of the NMPA stock solution were pipetted into the glass boat of the evaporation tube (cf. Fig. 2, Sect. 3.1). These volumes correspond to nitrosamine weights of 20 ng, 50 ng, 100 ng and 1 µg.
After the tube was attached to the denuder, ambient air was drawn through the apparatus at a flow rate of 8 L/min for 30 min. The NMPA or NEPA weights in the tube correspond to concentrations of 0.08–4.2 µg/m^3 for a 240 L air sample. The denuder was then closed and prepared and analysed as described in Sect. 3. The organic phase was concentrated to 200 µL. The procedure described was carried out six times and yielded the relative standard deviations and recoveries shown in the Table 1.

Table 1. Standard deviation (rel.) *s* and recovery.

Concentration µg/m³	Standard deviation (rel.) *s* %		Recovery rate	
	NEPA	NMPA	NEPA	NMPA
0.08	16	14	0.98	0.94
0.21	27	15	0.88	0.79
0.42	17	14	0.92	0.95
4.2	–	5	–	0.80

5.2 Quantification limit

The absolute quantification limit for NMPA or NEPA is 0.1 ng. It was determined according to DIN 32645 [1] (calibration curve method). The relative quantification limit is 0.05 µg/m³ NMPA or NEPA for a 240 L air sample, 250 µL sample solution and a 2 µL injection volume.

5.3 Selectivity

In the sampling system the named nitrosamines are selectively separated from the corresponding amines, which are not collected. Semi volatile nitrosamines (e. g. *N*-nitrosomorpholine, *N*-nitrosopyrrolidine or *N*-nitrosodibutylamine) are incompletely deposited in the denuder system under the conditions described here. The TEA detector system is selective in combination with gas chromatographic separation. As a result of the selectivity of the sampling system interference from other organic nitrogen compounds is not to be expected.

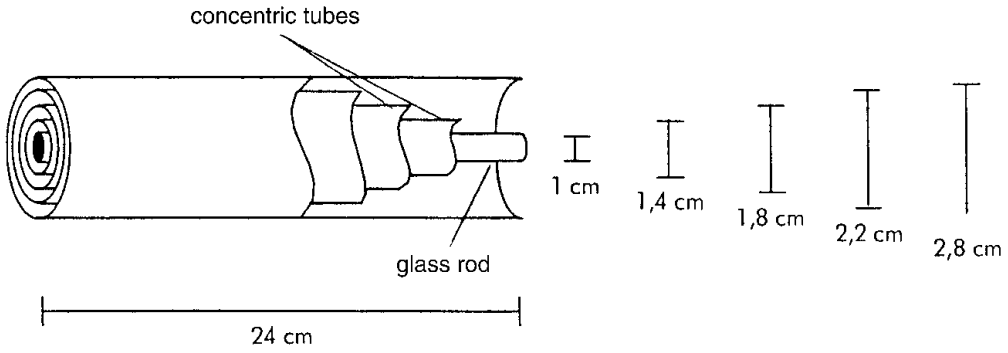
6 Discussion

The sampling of gaseous, aromatic nitrosamines in the annular denuder is restricted to a flow rate of 8 L/min and sampling for 30 minutes. Sampling is only possible at relative humidities below 50 %. At higher humidities recovery is reduced.
Under the sampling conditions described here, the corresponding amines are not deposited; therefore there is no artefactual formation of nitrosamines. Extensive laboratory experiments and investigations in practice have confirmed this [2, 3].
In addition to the two aromatic N-nitrosamines this procedure can also be used to determine semi volatile N-nitrosamines. For these compounds recovery is lower. Under the gas chromatographic conditions described, all components are completely separated.
With expected nitrosamine concentrations of >2.5 µg/m³ elution is possible without subsequent concentration of the solution.

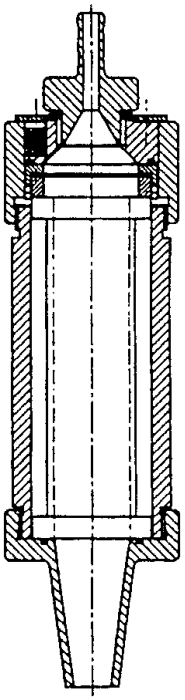
7 References

[1] DIN 32 645 (1994) Chemische Analytik – Nachweis-, Erfassungs- und Bestimmungsgrenze, Ermittlung unter Wiederholbedingungen. Beuth Verlag, Berlin.
[2] *Häger B, Nießner R* (1996) Determination of N-Nitrosomethylaniline and Methylaniline in the Gas Phase. Mikrochimica Acta 122:. 35–44.
[3] *Häger B, Breuer D* Ein neues Denuder-System zur Bestimmung von N-Nitrosomethylphenylamin und N-Nitrosoethylphenylamin in der Luft in Arbeitsbereichen. Gefahrstoffe-Reinhalt. Luft, in press.

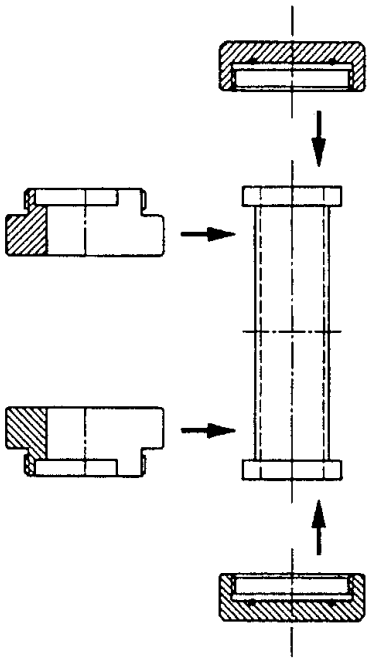
8 Appendix



Sketch: annular denuder



Sketch: denuder housing



Sketch: denuder with caps