



Health and Safety Executive
Occupational Medicine and
Hygiene Laboratory

MDHS 75

Methods for the
Determination of
Hazardous Substances

February 1993

Aromatic amines in air and on surfaces

Laboratory method using pumped acid-coated
filter, desorption and liquid chromatography

INTRODUCTION

Properties and uses

- 1 Aromatic amines are used as intermediates in the manufacture of plastics, dyes, drugs and carbamate pesticides. To date, this method has been validated for methylene dianiline (MDA), methylene bis orthochloroaniline (MbOCA), aniline and orthotoluidine.
- 2 MDA is used in the production of epoxy resins. The pure solid has a melting point of 92°C. It is very soluble in ethanol, benzene and ether.
- 3 MbOCA is used in the production of high performance polyurethanes. It is readily soluble in ethanol, ether and dilute acids, and slightly soluble in water. The commercial material can contain up to 10% polyamines and about 0.9% orthochloroaniline (OCA) and melts in the range 100 to 110°C. OCA is also formed by the decomposition of MbOCA above 200°C.
- 4 Aniline is a colourless, oily liquid, boiling point 184°C. It is used in the manufacture of dyestuffs, and is an intermediate in the manufacture of pharmaceuticals, photographic developers, plastics and ion-exchange resins.
- 5 Orthotoluidine is a colourless liquid with a boiling point of 200°C. In common with a number of aromatic amines, highly toxic fumes of NO_x are emitted on heating to decomposition.

Note: Acronyms used in this text:

- MDA : 4,4' Methylene dianiline
(diaminodiphenylmethane)
MbOCA : 4,4' Methylene bis orthochloroaniline
(2,2'dichloro 4,4' methylene dianiline)
OCA : orthochloroaniline

Toxicity

- 6 The aromatic amines listed in this MDHS are toxic by ingestion, inhalation and skin contact, and some are suspect human carcinogens. In many cases, the primary route of exposure is via dermal absorption.

Precautions

- 7 MbOCA is assigned the risk phase R45 'may cause cancer' and is therefore subject to the COSHH Carcinogens Approved Code of Practice. It is recommended, as good laboratory practice, that similar standards of control be applied to the other amines

listed. Decontaminate all contaminated glassware, surfaces and waste with Ehrlich's reagent before disposal.

First aid

- 8 In the event of personal contamination, remove contaminated clothing. Rinse the affected area with plenty of cold water and seek medical advice. In case of inhalation or ingestion, seek immediate medical attention. Do not induce vomiting.

Analytical methods

- 9 This is not an HSE 'reference' method in the strict analytical sense of the word. There are frequently several alternative methods for determining a particular analyte. With the exception of a few special cases, where an exposure limit is linked to a specific analytical method (eg for rubber fume or asbestos) the use of methods not included in the MDHS series is acceptable provided they have the accuracy and reliability appropriate to the application.

- 10 A preliminary screening method for MDA is described in Appendix A, for information.

PRINCIPLE

Air sampling

- 11 A measured volume of sample air is drawn through an acid-coated GF/A filter contained in a sampling head. A glass Tenax tube may be connected in series, to collect any of the more volatile aromatic amines which may pass through the filter, but is not required when sampling MDA, MbOCA or aniline.¹ The sampling efficiency for particulate matter may be compromised if the filter/tube combination is employed, as flow rates lower than 2 litres/min must then be used. After sampling, the filter is both neutralised and desorbed in a 0.135M sodium hydroxide solution in methanol/water (60:40). The Tenax tube is desorbed with methanol. The resultant solutions are analysed using a high performance liquid chromatograph equipped with an electrochemical detector, and quantified by comparison with a range of standard solutions.² An ultra-violet detector may be added to the system to increase selectivity.

Surface sampling

- 12 Surface contamination is estimated by means of a

wipe moistened with methanol, which collects a proportion of the amine present. The wipe is desorbed with methanol, and the resultant solution analysed.

SCOPE

13 The method described is for the determination of the time-weighted-average concentrations of aromatic amines in workplace atmospheres, and for the estimation of amine contamination on recently contaminated surfaces. The method is suitable for sampling over periods in the range 10 minutes to 8 hours, with a maximum recommended sampling volume of 200 litres.¹ Although described for the determination of personal exposure, the method may be used for fixed location air monitoring by suitable modification.

14 The method is expected to be suitable for the measurement of a range of airborne aromatic amines in a concentration range of at least 50 to 5500 µg/m³ for samples of 10 litres of air.¹ For 10 litre air samples, the following detection limits have been determined:

	µg/m ³
MDA	4
MbOCA	0.2
OCA	0.1
Aniline	3.8
Orthotoluidine	7.2

15 The mean analytical recovery of aniline from spiked filters was 100% (CV of 10.0%) for 214 µg/filter, and 107% (CV of 5.4%) for 32.1 µg/filter.¹ Recoveries for MDA, MbOCA, OCA and o-toluidine on filters, and for OCA on Tenax tubes, ranged from 91 to 106%. Recovery of MDA from spiked wipes was 81% (CV of 4%), but recovery from wiping a spiked glass surface was 55 to 60%.

16 The overall uncertainty in the determination of air samples is expected to be better than 25%,¹ within the working range of the method. This estimate assumes a sampling (pump) error of 5%.

17 Filters may be stored in sealed containers for up to 1 week without loss of sample.¹ Wipes, however, do not contain acid, which stabilises the amine on filters, and should be desorbed as soon as possible after sampling. The solutions are then stable at 4°C for several weeks.

Interferences

18 It is likely that methylene diphenyl diisocyanate (MDI) will hydrolyse to MDA on the acid-coated filters, thus giving a false high reading for MDA. The extent of isocyanate interference may be assessed by sampling and analysis according to MDHS 25, in parallel with sampling for MDA.

Quality control

19 The procedure described in paragraphs 38 to 40 acts as an internal quality control. This should be performed for every batch of filters, tubes or wipes. The results obtained should be comparable with those listed in paragraphs 14 to 16, which are described in detail in

an HSE report.¹ The performance meets the European Committee for Standardisation (CEN) performance criteria for analytical methods.⁴ These state that the overall uncertainty in a determination should be less than or equal to 50% for results between one-tenth and half of the Limit Value, and less than 30% for results between half and twice the Limit Value. Further information on quality assurance may be found in MDHS 71.

20 Recoveries from spiked filters and tubes should be better than 90% and analytical precision better than 10% CV. Recoveries from wipes are less reproducible and a correction for actual recovery (using a spiked blank) should be made with each batch of samples.

REAGENTS

During the analysis, use only reagents of recognised analytical reagent grade.

HPLC mobile phase

21 Dissolve 6 g of sodium dihydrogen orthophosphate and 1.43 g of disodium hydrogen orthophosphate in 1 litre of de-ionised water. Add 400 ml of this solution to 600 ml of methanol and mix thoroughly. De-gas by filtration under vacuum or with helium, then adjust the pH to 7.5 with sodium hydroxide solution or orthophosphoric acid. The composition of the mobile phase may be modified as necessary to achieve optimal separation or convenient retention times.

Filter desorbing solution

22 Dissolve 0.54 g of sodium hydroxide in 100 ml of de-ionised water. Add 40 ml of this solution to 60 ml of methanol and mix thoroughly.

Acid-coated filters

23 Place individual filters (Whatman GF/A 25 mm) on a clean glass plate about 5 to 10 mm apart. Add 200 µl of 0.135M sulphuric acid to each filter, taking care that the whole filter is coated and that none of the acid transfers between filters. Leave to dry at room temperature or in an oven at or below 100°C overnight. The filters may stick to the glass plate and are fragile when damp, so must be completely dry before handling. When dry, transfer the filters to a desiccator using tweezers, and store until required.

Ehrlich's reagent

24 Dissolve 2.5 g of p-dimethylaminobenzaldehyde in 1 litre of methanol to which 17 ml concentrated hydrochloric acid has been added. This solution is light-sensitive and should be stored in a refrigerator in dark glass until required, and used within 1 week.

APPARATUS

25 A suitable assembly for air sampling is illustrated in MDHS 14, Figure 1.³ The acid-coated filter (paragraph 24) is placed in a modified UKAEA sampling head with seven equispaced inlet holes. Other equivalent sampling heads may be used (criteria for judging equivalence of

other samplers is under discussion by standards organisations; on present information the IOM inhalable dust sampler will meet those requirements). The Tenax tube (paragraph 26), if used, is placed in a PTFE holder with a screw fitting, and connected to the sampling head (illustrated in MDHS 62, Figure 1).

26 NIOSH-style Tenax tubes may be used. These consist of a glass tube, 70 mm in length, with both ends flame sealed. The adsorbing section contains 30 mg of Tenax, and the back-up section 15 mg. The OSHA 'Versatile Sampler' (OVS) tube may also be suitable, but has not been evaluated by HSE for the present method. The tube is capable of sampling at a higher flow-rate than the Tenax tube, so may produce some improvement in sampling efficiency.

27 The pump should be suitable to be worn by a person carrying out normal work, and should be capable of running continuously for 8 h at the recommended flow rate (0.5 to 2 l/min). The total volume of air sampled by the pump over the sampling period should be within $\pm 5\%$ of the calculated volume. A flow-stabilised pump may be necessary to achieve this.

Wipes

28 'Regal' filmated swabs are recommended for surface sampling. These should be halved to give a convenient size for sampling and desorption.

High performance liquid chromatograph

29 An HPLC fitted with an electrochemical detector is suitable. The addition of an ultraviolet detector is advised, to increase confidence in peak identification for samples containing contaminants.

30 The sensitivity of the method is improved if temperature fluctuations are avoided, for example by thermostating the HPLC column and EC detector.

31 The proportion of methanol required in the mobile phase varies according to the aromatic amine being studied and the dimensions of the column in use. Chromatographic conditions that have been found to be suitable for MbOCA are:

Column dimensions	100 mm x 4.5 mm ID
Column packing	S3 ODS2
Column temperature	20°C
Mobile phase	60:40 methanol : buffer, pH 7.5 (paragraph 21)
Flow rate	0.8 ml/min
UV detector	242 nm
EC detector	Operating potential of + 0.7V (versus silver-silver chloride)

The retention time of MbOCA under these conditions is 10.4 minutes.

PROCEDURE

Calibration of sampling pumps

32 Measurement of the volume of air sampled may be

a significant source of error in the final calculation of aromatic amine concentrations. About 15 minutes before sampling is to begin, connect the pump to a filter holder (with a filter in place) by means of a flexible tube, and adjust the flow rate to the desired value by attaching a suitable calibrated airflow meter to the front of the filter holder. The pump should then be allowed to run for 15 minutes to stabilise the flow rate. Before taking the actual sample, a filter holder with a clean filter is fitted, with a Tenax tube if required, and the flow rate readjusted to its original value. The meter should also be used to check the flow rate at the end of the sampling period.

Collection of samples

Air samples

33 Connect the sampling assembly to the pump as described above. The filters should be handled with tweezers, and ends broken from the Tenax tubes carefully, to avoid damage to the 'o' ring seals. When used for personal sampling, the sampler should be mounted in the worker's breathing zone, for example on the lapel, with the filter surface approximately vertical. The pump is attached to the worker as appropriate to minimise inconvenience.

34 Draw a measured volume of air through the GF/A filter paper. The recommended air sample volume is 200 litres, and the sampling rate 2 l/min. Take a separate sample for each 100-minute period. If Tenax tubes are connected in series, the maximum achievable flowrate is 0.5 l/min, or 1 l/min if OVS tubes are used.

Surface samples

35 The area of each surface to be sampled should be measured. The surface should be wiped with a circular motion, going over the area three times, using a wipe moistened with 1 ml of methanol.

Blanks

36 Sample blanks should be prepared by using filters, Tenax tubes and wipes identical to those used for sampling, and subjecting them to the same handling procedure except for the actual period of sampling.

37 For transport back to the laboratory, seal the filters in individual tins and replace the end-caps on the Tenax tubes. Seal the wipe samples in individual glass bottles. Samples should be kept cool and analysed within 1 week.

Preparation of standards

38 Prepare a standard solution of each amine in methanol gravimetrically. Prepare further standard solutions by serial dilution of the first solution. Use these standards to spike acid coated filters, Tenax tubes and wipes from the same batch as the samples with a range of concentrations and to spike 2 ml aliquots of methanol to produce a similar range of calibration standards. The range used should exceed the concentration range expected for the samples. Also spike filters, tubes and wipes with a single level of amine in multiplicate (eg six of each) to estimate recovery.

Desorption

39 Desorb each filter in a sealed glass bottle containing 2 ml of the desorbing solution (paragraph 22) for 2 to 3 hours. Add 10 ml methanol to each wipe sample in a glass bottle, seal and leave for 3 hours to desorb, ensuring that the wipe is completely submerged. Shake well before removing an aliquot for analysis. Place each Tenax tube vertically in a suitable holder and add methanol to the top of the tube. Collect the first 2 ml of methanol washings from the tube.

Analysis

40 Desorb the spiked filters, tubes and wipes (paragraph 38) as described in paragraph 39. Inject into the liquid chromatograph a known fixed volume of each solution, in the range 5 to 50 µl. A standardised injection technique should be used, such that reproducible peak areas are obtained. Also inject the calibration standards, and construct a calibration graph. Check that recovery is consistent and close to 100% for filters and tubes, and that the response is linear. Inject the same volume of each sample solution. These may be compared with a single standard, provided the concentrations found remain within the established linear range.

CALCULATIONS

41 Calculate the concentration, in µg, of each aromatic amine in the sample by comparison with the standard. Calculate µg/m³ of amine in air as follows:

$$\text{Concentration of amine in air (}\mu\text{g/m}^3\text{)} = \frac{(m - m_{\text{blank}}) \times 1000}{V}$$

where m = weight (µg) of amine in sample (total of filter and tube)

m_{blank} = weight (µg) of amine in blank (total of filter and tube)

V = volume (litres) of air sampled

REPORT

42 Report concentration(s) of each aromatic amine in air to the nearest µg/m³, and µg total or µg per unit area for surface samples.

ADVICE

Advice on this method and the equipment used can be obtained from the Health and Safety Executive, Occupational Medicine and Hygiene Laboratory, Broad Lane, Sheffield S3 7HQ (tel: 0742 892000).

The Health and Safety Executive wishes, wherever possible, to improve the methods described in this series. Any comments that might lead to improvements would therefore be welcome and should be sent to the above address.

APPENDIX A

Screening method for MDA

Colorimetric analysis may be used as a preliminary

screening method for MDA. Any scanning or single beam spectrophotometer, or a filter photometer capable of measuring at the approximate absorbance maximum for MDA (590 nm), is suitable. Pumped samples are taken as described above, and a set of seven standards is prepared by spiking mixtures of 2 ml hydrochloric acid (0.5N) and 3 ml N,N-dimethylformamide (DMF) with 0 to 25 µg of MDA in methanol. The standards are allowed to stand for 10 minutes, then treated as described in MDHS 49, paragraph 36. The absorbances are measured at 590 nm using 1 cm cells, with de-ionised water as the reference. The sample filters are desorbed in 3 ml HCl solution, sonicated for 30 minutes, then filtered. 3 ml DMF is added to 2 ml of filtrate, and the solution treated as described for the standards. The concentration of MDA present is calculated by comparison with the standard solutions, and reported as mg/m³ of MDA in air as follows:

$$\text{concentration of MDA in air (mg/m}^3\text{)} = \frac{(A-B) \times 3}{V \times 2}$$

where A = weight (µg) of MDA on filter
B = weight (µg) of MDA in blank
V = volume (litres) of air sampled

REFERENCES

- 1 Hughes A and Cox R A Back-up data report on the evaluation of the MDHS method for sampling aromatic amines HSE Internal Report IR/L/IA/92/2
- 2 Purnell C J and Warwick C J Application of electrochemical detection to the measurement of 4,4'-methylenebis(2-chloroaniline) and 2-chloroaniline concentrations in air by high-performance liquid chromatography *Analyst* 105 (1980) 861-867
- 3 HSE *Methods for the determination of hazardous substances* (MDHS in series 1981-93) HMSO
- 4 CEN/TC137/WG2 Draft CEN standard PrEN 482 March 1991 General requirements for the performance of procedures for workplace measurements

LIST OF SUPPLIERS

OSHA 'Versatile Sampler' sorbent sample tube

SKC Ltd, Hamworthy Trading Estate, Dawkins Road, Poole, Dorset BH15 4JW (0202) 671121

'Regal' filmated swabs 7.5 cm x 7.5 cm, 8 ply

Surgicon Ltd, 84 Wakefield Road, Brighouse, W Yorks HD6 1QL

£2.00

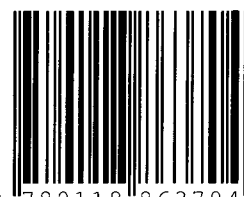
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