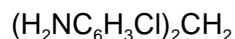


**MBOCA in urine****8302**

MW: 267.16

CAS: 101-14-4

RTECS: CY1050000

**METHOD:** 8302**EVALUATION:** PARTIAL**Issue 1:** 15 February 1984**Issue 2:** 15 August 1994**BIOLOGICAL INDICATOR OF:** exposure to 4,4'-methylenebis(2-chloroaniline)**SYNONYMS:** MOCA; di-(4-amino-3-chlorophenyl)methane; 4,4'-methylenebis(2-chloroaniline)

| SAMPLING  | MEASUREMENT   |
|---|---|
| <b>SPECIMEN:</b> 2 urines (pre- and post-exposure)                                    | <b>TECHNIQUE:</b> GAS CHROMATOGRAPHY, ECD   |
| <b>VOLUME:</b> 50 to 100 mL in polyethylene screw-cap bottles containing preservative | <b>ANALYTE:</b> heptafluorobutryl MBOCA derivative  |
| <b>PRESERVATIVE:</b> 3 mL of 30% (w/w) citric acid solution                           | <b>EXTRACTIONS:</b> MBOCA and analyte   |
| <b>SHIPMENT:</b> freeze urine and ship in dry ice in an insulated container           | <b>INJECTION VOLUME:</b> 1 µL   |
| <b>STABILITY:</b> stable for 2 days @ 25 °C and for 1 month @ -20 °C                  | <b>TEMPERATURE-INJECTOR:</b> 200 °C<br><b>-DETECTOR:</b> 300 °C<br><b>-COLUMN:</b> 1 min @ 90 °C; 35°/min; 4 min @ 250 °C |
| <b>CONTROLS:</b> collect urine from non-exposed workers                               | <b>COLUMN:</b> SE-54 capillary column, 30 m x 0.25-mm ID fused silica   |
|   | <b>CARRIER GAS:</b> 5% methane in argon, 40 mL/min  |
|   | <b>CALIBRATION:</b> control urine spiked with MBOCA; MDA as internal standard   |
|   | <b>QUALITY CONTROL:</b> frozen pooled urine; correct for creatinine content   |
|   | <b>RANGE:</b> 10 to 250 µg/L urine  |
|   | <b>ESTIMATED LOD:</b> 1 µg/L urine  |
|   | <b>RECOVERY:</b> 89% (100 µg/L of urine); 79% (4 to 25 µg/L urine)  |
|   | <b>PRECISION (<math>\bar{S}</math>):</b> 0.08   |
|   | <b>ACCURACY:</b> ± 27 to 37%  |

**APPLICABILITY:** MBOCA is commonly found in the urine of humans exposed to the compound in the plastic industry. Despite the extensive metabolism of MBOCA in the rat, analysis of the urine of exposed workers showed only MBOCA [1]. This GC-EC D method is useful in screening workers exposed to MBOCA.

**INTERFERENCES:** Imipramine has a similar retention time. A careful work history/questionnaire is suggested.

**OTHER METHODS:** This revises P&CAM 342 [2] and Method 8302 (dated 2/15/84). This method is 40-fold more sensitive than the method in the Special Hazard Review [3]. A similar, less sensitive method using GC-FID has been described [4].

**REAGENTS:**

1. 4,4'-Methylenebis(2-chloroaniline) (MBOCA) calibration stock solution, 200 mg/mL.\* Accurately weigh 50 mg MBOCA. Dissolve in a small volume of methanol. Dilute to 250 mL with methanol. Stable five days at 25 °C.
2. 4,4'-Methylenedianiline (MDA), internal standard, 10 µg/mL in methanol.
3. Hexane.\*
4. Diethyl ether.\*
5. Triethylamine (TEA), 0.05 M stock solution in hexane.
6. Heptafluorobutyric anhydride (HFBA).
7. Florisil, 60 to 100 mesh, activated by the manufacturer at 650 °C, heated to 130 °C for 24 h, then deactivated by adding 10% (w/w) water and mixing 2 h on a rotary mixer at 50 rpm. Stable one week.
8. Benzene.\*
9. NaOH.
10. KH<sub>2</sub>PO<sub>4</sub> buffer, 0.1 M, adjusted to pH 6 with conc. HCl.
11. Sodium sulfate, granular, anhydrous.
12. Methanol.
13. Citric acid, monohydrate, granular.
14. Pooled urine sample or Hycel urine control (Hycel Co., Houston, TX).
15. HCl, conc.
16. P-5 carrier gas, 5% methane in argon.

\* See SPECIAL PRECAUTIONS.

**EQUIPMENT:**

1. Bottles, polyethylene, screw-top, 125-mL.
2. Gas chromatograph with <sup>63</sup>Ni electron capture detector, integrator and capillary column (page 8302-1).
3. Pipets, Pasteur.
4. Centrifuge tubes, 15-mL, graduated, glass-stoppered.
5. Mixer, rotary, 50 to 60 rpm, for 20-mm culture tubes.
6. Culture tubes, 20 x 150-mm, screw cap, PTFE-lined.
7. Mixer, vibration.
8. Chromatography column, 7-mm ID x 20 cm, with a 25-mL reservoir and PTFE stopcock.
9. pH paper, wide range.
10. Nitrogen manifold for evaporating organic solutions.
11. Pipets, 10-mL.
12. Centrifuge, table top, clinical.
13. Syringes, glass, 10- and 50-µL.
14. Waterbath, 50 °C.
15. Volumetric flasks, 100- and 250-mL.
16. Glass wool.

**SPECIAL PRECAUTIONS:** Samples of urine collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle urine specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided. MBOCA, MDA, and benzene are suspect carcinogens; handle either in a glove box or a well-ventilated hood.

Handle ethyl ether and hexane with care; both are highly flammable and should be used in a hood.

### SAMPLING:

1. Collect 50 to 100 mL urine in a 125-mL polyethylene bottle containing 3 mL 30% citric acid.
2. Cap the bottle immediately after sample collection. Swirl gently to mix.
3. Ship the sample in dry ice in an insulated container.

### SAMPLE PREPARATION:

4. Thaw urine sample.
5. Perform a creatinine determination on an aliquot of the urine (e.g., [5]).
6. Pipet a 5-mL aliquot of urine into a clean culture tube.
7. Adjust pH to >12 using 10 N NaOH (ca. 0.1 mL).
8. Add 1 mL methanol. Mix well.
9. Add 5 mL 1:1 diethyl ether:hexane. Shake steadily for 2 min to extract the MBOCA. Centrifuge to separate phases. Transfer the organic phase by Pasteur pipet to a culture tube. Repeat with two additional 5-mL portions of 1:1 diethyl ether: hexane, combining the organic phases.  
NOTE: Emulsions may form; break them by adding methanol or by repeated centrifugation.
10. Concentrate the extract to ca. 1 mL under a gentle stream of nitrogen in a hood.
11. Add 50 µL TEA, 50 µL HFBA, and 25 µL MDA to the concentrated extract. Mix well in a hood.
12. Secure the cap loosely. Heat in a waterbath at 50 °C for 15 min.
13. Remove from waterbath. Add 2 mL hexane and 5 mL KH<sub>2</sub>PO<sub>4</sub> buffer. Mix well.
14. Centrifuge to separate phases completely. Transfer the organic layer containing the derivative, without disturbing the aqueous phase, with a Pasteur pipet into a 15-mL centrifuge tube.
15. If low concentration (<5 µg/L urine) is expected, use Florisil cleanup (see APPENDIX). Add 0.2 g Na<sub>2</sub>SO<sub>4</sub> and mix.

### CALIBRATION AND QUALITY CONTROL:

16. Prepare working standards over the range 0 (control) to 200 µg/L urine by adding aliquots of calibration stock solution to a control urine.
17. Extract 5 mL of each working standard (steps 6 through 15). Analyze the working standards with the samples (steps 20 and 21).
18. Prepare calibration graph (ratio of peak area of analyte to peak area of MDA vs. µg MBOCA/L urine).

### MEASUREMENT:

19. Set gas chromatograph according to manufacturer's recommendations and to conditions on page 8302-1.
20. Inject 1 µL hexane extract from step 15.
21. Measure the peak areas of the samples and internal standard. Divide the peak area of the sample by the peak area of the internal standard on the same chromatogram.

### CALCULATIONS:

22. Determine the MBOCA concentration in the urine sample, C<sub>u</sub> (µg/L), from the calibration graph.
23. Calculate the concentration of MBOCA/g creatinine in the urine sample, C (µg/g creatinine), using the creatinine value (C<sub>r</sub>) obtained in step 5.

$$C = \frac{C_u}{C_r}, \mu\text{g MBOCA/g creatinine.}$$

## GUIDES TO INTERPRETATION:

Linch, et al. [1] reported exposed workers having MBOCA at concentrations up to 370 µg/L of urine. No other reports have been found in the literature. CAL/OSHA has recommended that urinary MBOCA levels should not exceed 100 µg/L [6].

## EVALUATION OF METHOD:

Spiked urine specimens containing MBOCA concentrations of 4, 30 and 150 µg/L urine were analyzed in groups of 10 for each concentration. Precision ( $\bar{S}_r$ ) over the above analytical range was found to be 0.08 [2].

## REFERENCES:

- [1] Linch, A. L., G. B. O'Connor, J. R. Barnes, A. S. Killian, and W. E. Neeld. Methylene-bis-Ortho-Chloroaniline (MOCA): Evaluation of Hazards and Exposure Control, Am. Ind. Hyg. Assoc. J., **32**, 802-819 (1971).
- [2] NIOSH Manual of Analytical Methods, 2nd ed., Vol. 7, P&CAM 342, U.S. Department of Health and Human Services, Publ. (NIOSH) 82-100 (1981).
- [3] Special Hazard Review with Control Recommendations for 4,4'-Methylenebis-(2-Chloroaniline), U.S. Department of Health, Education, and Welfare, Publ. (NIOSH) 78-188 (1978).
- [4] Van Roosmalen, P. B., A. L. Klein, and I. Drummond. An Improved Method for Determination of 4,4'-Methylenebis-(2-Chloroaniline) (MOCA) in Urine, Am. Ind. Hyg. Assoc. J., **40**, 66-69 (1979).
- [5] Tietz, N. W. Fundamentals of Clinical Chemistry, 2nd ed., pp. 994-999, W.B. Saunders, Co., Philadelphia, PA (1976).
- [6] California Occupational Safety and Health Administration (CAL/OSHA), Title 8, Sec. 5215; General Industry Safety Orders, 4,4'-Methylenebis(2-chloroaniline), Register 81, No. 22 (May 30, 1981).

## METHOD WRITTEN BY:

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## APPENDIX: FLORISIL CLEANUP AND ANALYSIS (for urine containing <5 mg MBOCA/L)

1. Concentrate the extract (the organic layer from step 14) to approximately 0.5 mL under a gentle stream of nitrogen at 30 °C in a waterbath.
2. Place a small glass wool plug in a chromatographic column. Add 1.6 g of 10% deactivated Florisil. Top with 2 cm of anhydrous Na<sub>2</sub>SO<sub>4</sub>.
3. Pre-rinse the packed column with 10 mL hexane.
4. Transfer the 0.5 mL of derivatized extract to the column with a Pasteur pipet. Add the extract just as the level of hexane rinse is even with the Na<sub>2</sub>SO<sub>4</sub> layer.
5. Quickly rinse the extract tube with several additional small quantities of hexane. Do not allow the level of rinse to fall below the Na<sub>2</sub>SO<sub>4</sub> layer.
6. Discard the eluted pre-rinse.
7. Elute the column with 10 mL of 40% benzene/hexane (v/v). The solvent mixture should not fall below the Na<sub>2</sub>SO<sub>4</sub> layer; discard the eluate.
8. Elute the column with 10 mL of 100% benzene. Save the eluate and concentrate to 1 mL.
9. Inject 1 µL of the concentrated benzene fraction into the GC that has been set to the conditions described on page 8302-1.