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# Determination of Urinary 2-Ethoxyacetic Acid as an Indicator of Occupational Exposure to 2-Ethoxyethanol

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A biological monitoring method which can detect human exposure to glycol ethers is described. A procedure for measurement of 2-ethoxyacetic acid (EAA), a urinary metabolite of 2-ethoxyethanol (EE), has been validated. EAA is removed from the urine specimen by a methylene chloride extraction of an EAA-tetrabutyl ammonium hydrogen sulfate ion pair. The ion pair subsequently reacts with pentafluorobenzyl bromide to produce the pentafluorobenzyl derivative of EAA. The EAA derivative is separated from other co-extracted urinary constituents by packed column gas chromatography and quantitated with flame ionization detection. 2-Butoxyacetic acid and 2-methoxyacetic acid can also be separated by this procedure. The analytical range for EAA is 5 to 100 μg/ml of urine; the limit of detection is 4 μg/ml, while the limit of quantitation is 7 μg/ml. The day-to-day relative standard deviation (Sr) was better than 4.7 percent; the corresponding within-day Sr was less than 2.0 percent. The procedure has been applied to urine specimens collected from shipyard workers exposed to paints containing 2-ethoxyethanol. Preliminary results indicate this procedure can detect human occupational exposure to EE. **Smallwood, A.W.; DeBord, K.; Burg, J.; Moseley, C.; Lowry, L.: Determination of Urinary 2-Ethoxyacetic Acid as an Indicator of Occupational Exposure to 2-Ethoxyethanol. Appl. Ind. Hyg. 3:47–50; 1988.**

## Introduction

The glycol ethers, 2-methoxyethanol (ME), 2-ethoxyethanol (EE), and 2-butoxyethanol (BE), are widely used in many industrial processes and consumer products. Recent animal studies in rats, mice, and rabbits indicate ME and EE are associated with a number of adverse reproductive effects. The effects observed included embryotoxicity, fetal death, birth defects, and male infertility.[1–3] Although the adverse reproductive effects seen in animals have not been reported in humans, these animal studies suggest that exposure of pregnant women to ME and EE should be minimized.[4–5] These animal studies have prompted a leading manufacturer of glycol ethers and The American Conference of Governmental Industrial Hygienists (ACGIH) to recommend that the ambient air TWA be less than 5 ppm of ME or EE and that skin contact be avoided.[4–6] The National Institute for Occupational Safety and Health (NIOSH) recommends that ambient air exposures should be reduced to the lowest feasible level and that skin contact be prevented.[7] Although the control of air levels can reduce exposure, consistent or repeated dermal contact with ME, EE, and BE can result in a significant exposure.[4–5,8–10] ME, EE, and BE are absorbed and metabolized in a variety of animal species to 2-methoxyacetic acid (MAA), 2-ethoxyacetic acid (EAA), and 2-butoxyacetic acid (BAA) which are excreted in the urine. These acid metabolites represent the major urinary metabolites of the respective glycol ethers.[8–9,11–12]

A method for the determination of MAA and EAA in urine utilizing extraction of freeze-dried urine, derivatization with diazomethane, and separation by capillary gas chromatography using flame-ionization detection has recently been described.[11] Although the method appears to be useful for assessment of EAA and perhaps MAA in urine, it requires considerably more sample preparation steps than the method described in this paper and utilizes a potentially dangerous derivatization reagent.

The procedure described in this paper uses phase-transfer catalysis to extract and derivatize EAA in urine specimens, a procedure that eliminates multiple extractions and combines the extraction and derivatization steps into one operation. The fully ionized EAA is extracted from the urine specimen into methylene chloride as an association complex with tetrabutylammonium-hydrogen sulfate.[13–14] The complex reacts with pentafluorobenzyl bromide to form a derivative which is quantitated by gas-liquid chromatography using flame ionization detection. This procedure has been validated and applied to urine samples collected during field studies. The procedure also can detect BAA and MAA in urine; however, some matrix effects prevent quantitation at low levels. Extensive field validation has not been done for humans exposed to BAA and MAA.

## Material and Methods

### Chemicals

MAA, EAA, and tetrabutylammonium-hydrogen sulfate came from Aldrich Chemical Co. (Milwaukee, WI). Pentafluorobenyzl bromide was supplied by Pierce Chemical Co. (Rockford, IL). High purity methylene chloride was obtained from Burdick and Jackson (Muskegon, MI); analytical reagent grade potassium carbonate was supplied by Mallinckrodt, Inc. (Paris, KY). BAA was synthesized by the procedure outlined for EAA and purified by preparative liquid chromatography.[15]

### Collection of Urine Samples

Individual voiding (spot) urine samples were collected in 100-ml polyethylene wide-mouth bottles. After collection, an aliquot of each urine sample was transferred to a 20-ml scintillation vial and kept frozen at −20°C until analyzed.

### Extraction and Derivatization

One gram of potassium carbonate, 2.0 ml of urine, 1.5 ml of 0.2 M tetrabutylammonium-hydrogen sulfate, 2.0 ml methylene chloride, and 100 µL of pentafluorobenzyl bromide were added to a 16 × 100 mm screw-capped culture tube. The culture tube was placed on a mixer and rotated at 60 rpm for 2 hours. The culture tube was then placed in a 50°C water bath for 20 minutes. After removal from the water bath, the sample was again rotated on the mixer for one hour. After the layers separated, the upper aqueous layer was removed with a disposable Pasteur pipet and discarded. The lower methylene chloride phase was washed and rotated twice with separate 3.5 ml portions of distilled water to remove unreacted reagents. The upper aqueous layer was removed, and the emulsified methylene chloride layer centrifuged at 2800 rpm for 5 minutes to remove water. The upper aqueous layer was removed. Anhydrous sodium sulfate was added to the remaining methylene chloride layer until the emulsion cleared. The methylene chloride layer was then placed into a 1-ml autosampler vial.

### Chromatographic Conditions

The gas chromatograph (Hewlett Packard 5890A with a flame ionization detector) was equipped with an auto-sampler (Hewlett Packard 7671A) and a sample event control module (Hewlett Packard 19405A). Separation was on a glass column (1.83 m length × 4 mm internal diameter) packed with 4 percent SE-30 and 6 percent OV-210 on 100/120 mesh Chromosorb WHP. Nitrogen was used as the carrier gas at a flow of 55 ml/min. The column was operated at 160°C for 7.2 minutes, then programmed at 35°C/min to 220°C, followed by a 6-minute hold, a 5-minute cool down, and a 2-minute equilibration at 160°C. The injector port and detector had respective temperatures of 190° and 275°C. The volume injected was 4 µL. Quantitation was done by measurement of the peak area using the Hewlett Packard 3392A integrator.

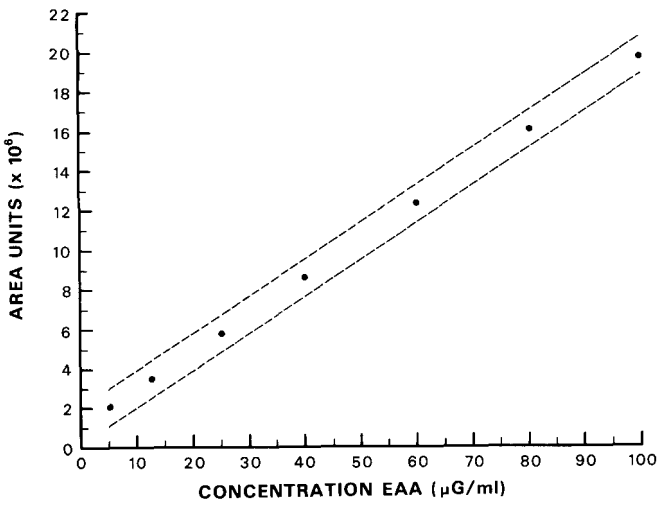


**FIGURE 1.** Calibration curve for EAA in urine. The dotted lines represent the 95% confidence bands of predicted concentrations (Y = 1.099 + 0.1811 X).

### Calibration

Stock solutions of EAA, MAA, and BAA were prepared by adding 0.2 g of each acid to a 200-ml volumetric flask and diluting to the mark with urine pooled from nine adult males not exposed to glycol ethers. Working standards were prepared by diluting portions of the stock standard with pooled urine to produce concentrations of 5, 12.5, 25, 40, 60, 80, and 100 µg/ml. Triplicate portions of these working standards, along with pooled urine with no added EAA, MAA, or BAA, were extracted, derivatized, and analyzed each day for five days. Linear regression analysis was used to establish a calibration curve and the 95 percent confidence bands for the predicted values over a concentration range of 5 to 100 µg/ml.[16,17]

### Sample Analysis and Quality Control

Urine samples from workers exposed to paints containing EE were analyzed by the described extraction and derivatization procedure. Quality control samples were prepared by spiking blank urine at several different concentrations. Small volumes of these quality control samples were kept frozen for later analysis. A frozen aliquot was thawed and included in each batch of samples. If the values for the quality control samples fell within the predicted 95 percent confidence limits for that concentration, the run was accepted and the data utilized.

Urine samples from workers were also analyzed for creatinine using an automated version of the classic Jaffee reaction to correct for dilution of the urine.

### Description of Workplace

Thirty-two workers from a shipyard painting operation who applied paint with spray and brush were evaluated for exposure to EE. Twenty shipyard workers not exposed to glycol ethers served as controls. Some of the painters' working environments were in confined spaces below deck, while others were in the open. Protective clothing and respirators were worn by some of the painters. Personal breathing zone samples were collected with pre- and post-shift urine samples for three consecutive days. Urine samples from each worker were collected daily for one week at the beginning and end of each workday. A total of 280 urine samples were collected over the work week from 52 workers.

## Results and Discussion

### Characteristics of the Method

Figure 1 shows the calibration curve for EAA added to pooled human urine. The calibration curve is linear over the range of 5 to 100 µg/ml. The 95 percent confidence bands for the true predicted concentration are also shown. The limit of detection (LOD), defined as the mean of the blank response plus 3 standard deviations, was 4 µg/ml. The limit of quantitation (LOQ), defined as the mean of the blank responses plus 10 standard deviations, was 7 µg/ml.

Figure 2 shows chromatograms of a pooled urine sample from unexposed humans and the same urine sample to which was

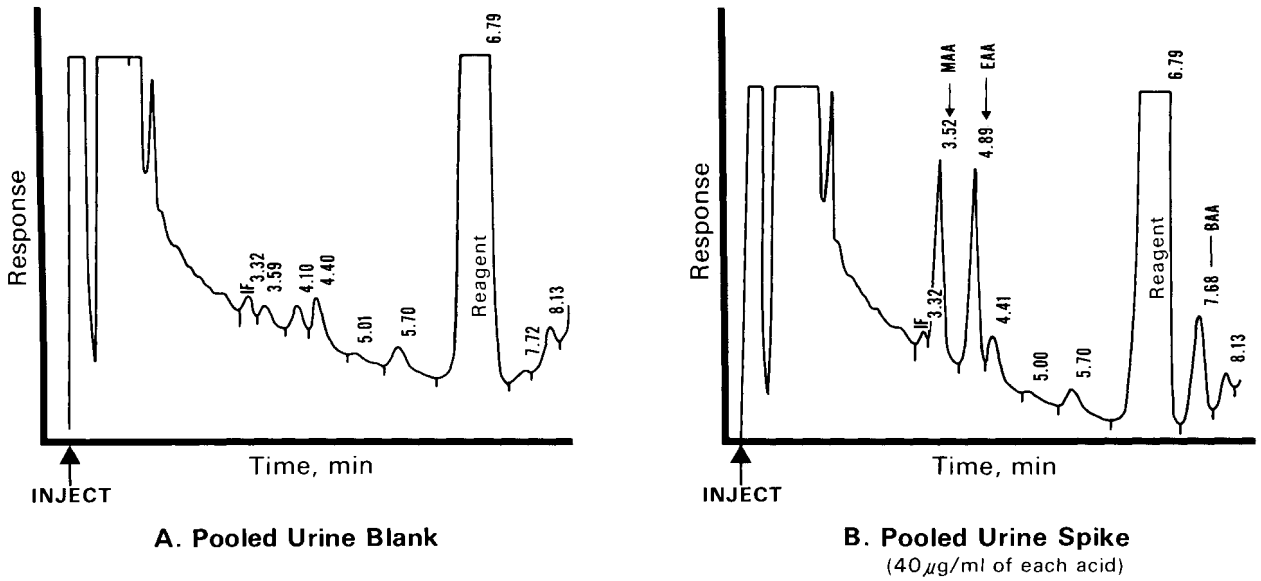


**FIGURE 2.** Chromatograms of (a) pooled urine with no added EAA and (b) pooled urine with 40 μg/ml of MAA, EAA and BAA added.

added 40 μg/ml each of EAA, MAA, and BAA. Note that MAA, EAA, and BAA are resolved. The large peak at a retention time of 6.79 minutes is a by-product of the derivatization reaction.

Table I shows both the day-to-day variation of EAA, added to pooled urine from unexposed humans, over a five-day period and the within-day variation. The day-to-day precision was based on 15 values obtained, 3 per day for 5 days. The within-day precision was based on the average of 3 values obtained on day 5. Results show that the day-to-day relative standard deviation ranged from 3.0 to 4.7 percent. The within-day relative standard deviation was less than 2 percent over the entire range.

EAA added to pooled urine was stable for at least eight months at −20°C. Samples of frozen, spiked, pooled urine were analyzed after eight months and the results compared to those from freshly prepared specimens. The results from the frozen specimens did not differ significantly from the freshly prepared samples.

**TABLE I. Within Day and Day-to-day Variation in EAA Added to Pooled Urine**

| EAA Conc. μg/ml | Mean Peak Area* | Standard Deviation (S) | Relative S (Sr) % |
|---|---|---|---|
| | **Day-to-Day, n = 15** | | |
| 5.0 | 2.09 | 0.094 | 4.5 |
| 12.5 | 3.46 | 0.141 | 4.1 |
| 25.0 | 5.74 | 0.205 | 3.6 |
| 40.0 | 8.64 | 0.257 | 3.0 |
| 60.0 | 12.10 | 0.389 | 3.2 |
| 80.0 | 16.20 | 0.769 | 4.7 |
| 100.0 | 19.82 | 0.930 | 4.7 |
| | **Within Day, n = 3** | | |
| 5.0 | 2.10 | 0.015 | 0.7 |
| 12.5 | 3.51 | 0.021 | 0.6 |
| 25.0 | 5.85 | 0.097 | 1.7 |
| 40.0 | 8.79 | 0.145 | 1.6 |
| 60.0 | 12.29 | 0.227 | 1.8 |
| 80.0 | 16.20 | 0.268 | 1.6 |
| 100.0 | 19.92 | 0.103 | 0.5 |

*Peak area units from the HP 3392A integrator

In order to determine if there was a possible urine matrix effect on the EAA method, nine individual urine samples from unexposed humans were spiked with 80 μg/ml of EAA. Each specimen was analyzed in duplicate and the results compared. Results showed that the relative standard deviation of the area response for all nine specimens (18 analyses) was less than 9.1 percent, showing little effect of individual matrices on the EAA analytical results.

## Use of the Method to Quantitate EAA in Urine of Workers

EAA in urine is an indicator of worker exposure to paints containing EE. Table II shows the maximum levels of EAA, expressed as mg EAA/g creatinine, found in any single urine specimen from unexposed shipyard workers (n = 20), painters not using paints containing EE (n = 5), and painters using paints containing EE (n = 27). The mean values show marked differences in maximum EAA values. The low levels of EAA seen in workers not using paints containing EE probably reflects usage of EE containing paints in the work area by others. The wide range of EAA levels seen in painters using EE-containing paints probably is due to considerable variation in work assignments, such as spray vs. brush painting, location of work area, and use of personal protective equipment.

Figure 3 shows sample chromatograms of urine samples from two exposed workers. The concentration of EAA shown in (a) is 16 μg/ml and in (b) is 36 μg/ml, which correspond to respective creatinine-corrected concentrations of 9 and 13 mg EAA/g creatinine.

The data described in this paper are preliminary and show EAA in the urine of workers exposed to paints containing EE. A

**TABLE II. Urinary Ethoxyacetic Acid (EAA) in Workers Exposed to 2-Ethoxyethanol (EE)**

| Group, (number of workers) | Maximum EAA mg/g creatinine* |
|---|---|
| Controls, shipyard workers (n = 20) | Not detected |
| Painters, not using EE (n = 5) | 6.6 ± 3.91 |
| Painters, using EE (n = 27) | 25.0 ± 20.7 |

*Results, shown as the mean ± 2 standard deviations, are calculated using the maximum level of EAA seen in any single voiding from an individual worker.

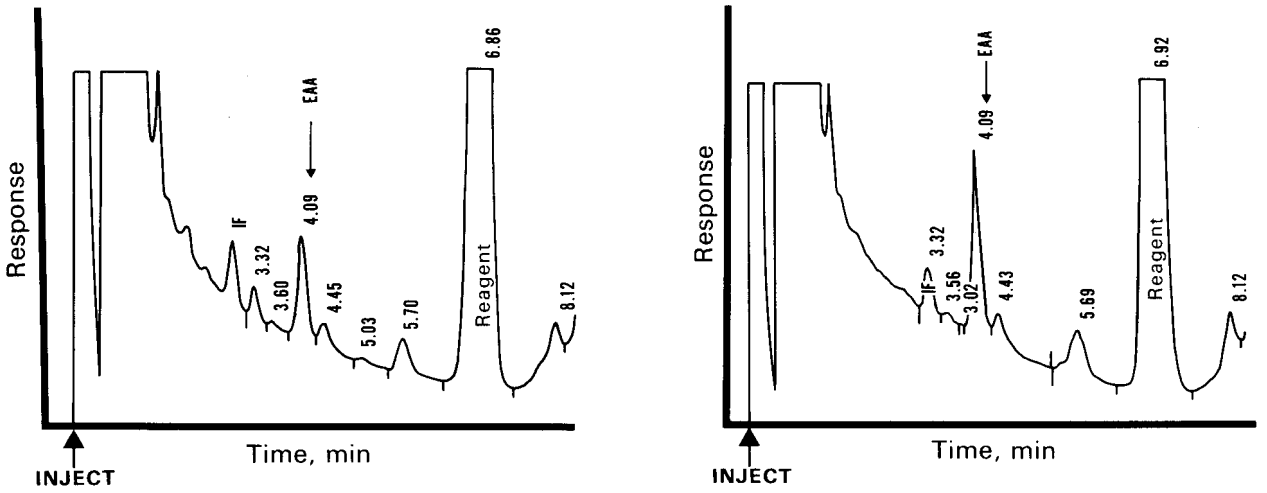


**FIGURE 3.** Chromatograms of two painters exposed to paints containing EE. Concentrations of EAA are 16 μg/ml in (a) and 36 μg/ml in (b).

companion paper is in preparation which will present the industrial hygiene data and will explore the possible relationships between exposure, work practices, environmental conditions, paint type, and paint usage with the levels of urinary EAA.

### Applicability of the Method to Quantitate BAA and MAA

The method appears to be applicable for quantitation of BAA. However, screening of the field samples indicated no exposure to BE; therefore, extensive method validation was not done. Preliminary evaluation of a possible matrix effect, performed as described for EAA, showed a relative standard deviation of less than 7 percent indicating little or no matrix effect. LODs and LOQs, although not formally established, were in the same range as those determined for EAA.

On the other hand, a large matrix effect was observed when the method was applied to MAA. The matrix effect experiment showed a relative standard deviation of 26 percent, an unacceptable sample-to-sample matrix effect. Further research is needed to resolve the matrix effect problem and validate the method for MAA.

## Recommendations

No final recommendations for use of this method for the measurement of EAA can be made to the practicing industrial hygienist based on the data presented in this paper. This paper describes a measurement technique for EAA and presents preliminary data indicating that the measurement technique has the potential for assessing EE exposure in painters who use EE containing paints. This validated method can be recommended for use in research studies designed to assess the relationship between exposure, measured by traditional industrial hygiene methods, and worker uptake, as measured by biological monitoring. Summary data shown in Table II suggest that EAA measurements can be of value. Research on the possible relationship between work practices, airborne exposure, and EAA in urine is continuing in our laboratories.

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