The University of Michigan

SCHOOL OF PUBLIC HEALTH
DEPARTMENT OF ENVIRONMENTAL
AND INDUSTRIAL HEALTH

109 OBSERVATORY STREET
ANN ARBOR, MICHIGAN 48109-2029
U.S.A.

October 17, 1996

Centers for Disease Control & Prevention
Grants Management Branch
ATTN: Karen E. Reeves
255 East Paces Ferry Road, NE
Mail Stop E-01
Atlanta, Georgia 30305

Re: 5 R01 OH03024-02
Biological Monitoring of Methanol Exposure

Dear Ms. Reeves:

As per the letter of 09 September 1996 from Ms. Georgia L. Jang, I am pleased to submit a final performance report for the above captioned project.

All of the original objectives were achieved. We also were able to address additional hypotheses, as described in the attached report.

I wish to indicate that there were no inventions conceived in conjunction with this project.

Please contact me if you have any questions.

Sincerely,

Alfred Franzblau, MD
Associate Professor of Occupational Medicine
Principal Investigator

enclosures

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB98-137755

4. Title and Subtitle Biological Monitoring of Methanol Exposure, Final Performance Report, October 17, 1996

5. Report Date 1996/10/17

6.

7. Author(s) Franzblau, A.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address

10. Project/Task/Work Unit No.

11. Contract (C) or Grant(G) No.

(C)

(G) R01-OH-03024

12. Sponsoring Organization Name and Address Department of Environmental and Industrial Health, School of Public Health, University of Michigan

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words) Reproducible and noninvasive methods for biologically monitoring occupational methanol (67561) exposures were developed, and the impact of exercise and cutaneous methanol exposures on such indices was evaluated. Formic-acid (64186) in urine was used as a quantitative biological exposure indicator for exposure of humans to methanol via the inhalation route and via the cutaneous route. Methanol in urine was evaluated as a quantitative biological exposure indicator for human exposures to methanol. The use of methanol in end expired air was evaluated as a quantitative biological exposure indicator. The effects of exercise, monitored by the ventilation rate, on formic-acid in urine, methanol in urine and methanol in end expired air were evaluated in humans exposed to methanol via the inhalation route. Formic-acid was a very poor biological indicator of methanol exposure in the range of the permissible exposure and threshold limit values. Regarding the use of methanol in urine as a biological index indicated that all methanol based parameters were approximately linearly related to exposure concentrations, but there was considerable inter-individual variations in all measured parameters. Blood and breath concentrations of methanol were disproportional for varying periods of time during and following cessation of methanol exposure, depending on the route of exposure. In settings where both dermal and inhalation exposures can occur, it would be necessary to wait at least 2 hours to collect breath specimens following cessation of methanol exposure.

17. Document Analysis **a. Descriptors**

b. Identifiers/Open-Ended Terms NIOSH-Publication, NIOSH-Grant, Grant-Number-R01-OH-03024, End-Date-07-31-1996, Grants-other, Biological-monitoring, Alcohols, Inhalation-studies, Skin-exposure, Skin-absorption, Urinalysis

c. COSATI Field/Group

18. Availability Statement

19. Security Class (This Report)

21. No. of Pages 5

22. Security Class (This Page)

22. Price

(See ANSI-Z39.18)

OPTIONAL FORM 272(4-77)
(Formerly NTIS-35)

The Final Performance Report for the Project Entitled
"Biological Monitoring of Methanol Exposure" (5 R01 OH03024-02)

The original goals and objectives for this project were to identify valid, reproducible and noninvasive methods for biologically monitoring occupational methanol exposure, and to assess quantitatively the impact of exercise and cutaneous methanol exposure on such indices. The underlying approach was to perform a series of controlled experiments with volunteer subjects. Specific objectives were to investigate the:

1. Use of formic acid (formate) in urine as a quantitative biological exposure indicator for exposure of humans to methanol via the inhalation route and via the cutaneous route;

2. Use of methanol in urine as a quantitative biological exposure indicator for exposure of humans to methanol via the inhalation route and via the cutaneous route;

3. Use of methanol in end-expired (alveolar) air as a quantitative biological exposure indicator for exposure of humans to methanol via the inhalation route and via the cutaneous route;

4. Effect of exercise, as measured by the ventilation rate, on formic acid in urine, methanol in urine and methanol in end-expired air when humans are exposed to methanol via the inhalation route.

The data and analyses from this study permit separate examination of the major factors which are believed to influence biological indicators of methanol exposure in the occupational setting: airborne concentration of exposure; duration of exposure; level of pulmonary ventilation; and cutaneous exposure.

Item #1 is addressed in paper 4 in the reference list. Overall, regardless of whether exposure is inhalation or dermal, formic acid in urine is a very poor biological indicator of methanol exposure in the range of the permissible exposure limit (PEL) and threshold limit value (TLV) (200 PPM). In fact, the ACGIH rescinded their formic acid based BEI in late 1994, based in part on data derived from our investigations.

Item #2, use of methanol in urine as a biological index of exposure, is addressed in papers 4 and 6. The following biological determinants were examined: total methanol excreted during the 'shift'; mean concentration of methanol excreted during the 'shift' (uncorrected, and corrected for specific gravity (SpGr) and creatinine (Cr)); and also the concentration of methanol excreted in urine immediately following cessation of exposure (again, uncorrected, and corrected for SpGr and Cr). All methanol-based parameters were approximately linearly related to exposure concentration. However, there was considerable inter-individual variation in all measured parameters. Because of large inter-individual variation, methanol in urine (i.e., total excreted during the shift, concentration at end of shift or mean concentration excreted across shift) do not appear suitable for quantitative assessment of individual exposures, but methanol in urine does appear to be useful as a semiquantitative, or qualitative index of individual exposure, or to assess quantitatively methanol exposure of a group of workers with similar exposures.

Item #3, use of methanol in end-expired (alveolar) air as a quantitative biological exposure indicator for exposure of humans to methanol via the inhalation route and via the cutaneous route, is examined in papers 1, 3 and 6. A fundamental assumption of monitoring breath for a toxicant is that the concentration of the toxicant in breath is proportional to the concentration in blood. The present study was designed, in part, to assess the conditions under which measurement of methanol in breath would be useful for estimating the blood concentration of methanol following inhalation or dermal exposures to methanol. Paid volunteer subjects underwent controlled inhalation exposure to methanol vapor at various concentrations for 8 hours, or dermal exposures (without inhalation exposure) to methanol for varying periods of time. Blood and end-expiratory air were analyzed for methanol from samples obtained prior to exposures, and at various times during and after exposures. The results demonstrate that blood and breath concentrations of methanol are disproportional for varying periods of time during and following cessation of methanol exposure, depending on the route of exposure (dermal versus inhalation). In settings where there might be opportunities for inhalation and dermal exposure to methanol, it would be necessary to wait at least two hours to collect breath specimens following cessation of methanol exposure. This prolonged waiting period might serve to decrease the attractiveness of measurement of methanol in breath as a strategy for monitoring occupational methanol exposure.

Item #4, the effect of exercise, as measured by the ventilation rate, on formic acid in urine, methanol in urine and methanol in end-expired air when humans are exposed to methanol via the inhalation route, was examined in paper 4. Subjects were exposed to vapor concentrations of 0, 100, 200, and 400 parts per million (PPM) during separate 8-hour sessions. Subjects repeated each exposure twice: once while sedentary and once while performing light intermittent exercise on a bicycle ergometer. Except for a 15 minute interruption after 6 hours, exposures lasted 8 hours. Urine samples were obtained pre-exposure and immediately following cessation of exposure after 8 hours, and all urine was collected during exposure sessions. Breath and blood samples were also collected.

The exercise protocol was designed to increase mean minute ventilation 50% over baseline sedentary ventilation. During each exposure session, two subjects alternated every 30 minutes on a bicycle ergometer. Minute ventilation was first measured at rest. Subjects then began cycling on the ergometer. After subjects accommodated to using the ventilation test equipment while cycling (about 5 or 10 minutes), cycling work load was adjusted to achieve a minute ventilation rate that was 100% over baseline. Ventilatory monitoring was continued for the duration of the first exercise period only. During subsequent 30 minute exercise periods, subjects exercised at the previously determined work load on the ergometer. No direct measurements of minute ventilation were performed after the first period of exercise because of the discomfort associated with breathing through a mouthpiece for prolonged periods. Once set, the ergometer automatically maintains the set workload regardless of cycling speed. Since subjects alternated periods of rest (baseline ventilation) with equal periods of exercise (ventilation 100% over baseline), the overall mean ventilation rate during exposure sessions with exercise was estimated to be 50% over baseline.

The mean concentrations of methanol in urine (uncorrected for SpGr or Cr) collected following exercise were consistently higher than the corresponding sedentary values for non zero exposures. Previous studies have shown that methanol absorption is linearly related to ventilation rate, and most of the same subjects participated in all exposure sessions (thus largely controlling for inter-individual biological differences among subjects studied in each session), we anticipated that the values with exercise would be 50% above the corresponding sedentary values. The actual

mean results with exercise were generally in the range of 50% over corresponding sedentary results (76.7% for 100 PPM, 25.7% for 200 PPM, and 51.8% for 400 PPM), suggesting that subjects maintained roughly their 'target' cycling speeds (and ventilation rates) when exercising. These results essentially confirm previous studies which examined the effect of ventilation rate on inhalation methanol absorption.

In addition to the four original objectives, we also examined how various methods and durations of storage of urine and blood specimens might impact on subsequent laboratory analyses for methanol (see paper 2). The study was designed to test the stability of methanol in blood and urine samples stored at 4°C and -20°C for various periods of time up to 7 months. Methanol recoveries of the stored blood samples were found to fit a first order decay model, with the best estimates for the half-life of methanol in chilled and frozen blood of 114 ± 14 and 240 ± 58 days, respectively. A half-life of 562 ± 145 days is estimated for chilled and frozen urine. These long half-lives enhance the utility of methanol bioindicators. While freezing increased recovery in blood, it also decreased the reproducibility of results. Thus, refrigeration of samples is recommended if the analysis will be completed within about one month of sample collection, and freezing of samples is suggested otherwise. For blood, sample preservation was not enhanced using an all-glass storage system; a conventional sampling container yielded equivalent results.

In a series of studies related to the dermal exposures to methanol, we also studied the possible utilization of airborne emissions of methanol at skin surfaces as a possible biological exposure index (see paper 3). Dermal exposures to methanol were administered in a clinical study designed to compare several biological indicators. Four subjects were exposed in five exposure sessions of varying length. In each session, a sequence of measurements of methanol concentrations in blood, breath, and headspace samples of air at exposed and unexposed skin were collected before and after dermal exposures. Skin headspace samples, collected in gas sampling bags, were designed to reflect equilibrium skin:air partitioning. At exposed skin, headspace samples were highly elevated for at least 8 hours following exposure, indicating the presence of a methanol reservoir in skin. After exposure, methanol concentrations at exposed skin showed a rapid initial decline, then a slower first-order decrease. Methanol concentration s were clearly detectable in headspace samples at unexposed skin. Substantial transfer from exposed skin occurred due to mechanical contact and washing. When transfer was restricted, surface concentrations at unexposed skin were similar to levels in breath and were strongly correlated to methanol concentrations in blood. While results are preliminary due to the small samples sizes and several unresolved experimental issues, the simple, rapid, and noninvasive skin headspace measurements appear useful as a biological exposure indicator that clearly shows the presence and site of dermal exposure, and measurements at unexposed skin reflect concentrations in blood.

We also performed a series of short-duration inhalational methanol exposures (paper 6). Due to their transient nature, short-term exposures of toxic compounds can be difficult to detect and quantify using environmental or occupational monitoring techniques. Biological exposure indexes (BEIs) may be applicable in such applications and may provide a convenient way to estimate important toxicological parameters. We investigated relationships between methanol concentrations in the blood, urine and breath of volunteers exposed to methanol vapor at 800 PPM for periods of 30 minutes, 1, 2 and 8 hours. Results indicate several factors which must be considered to interpret BEIs. Concentration of the BEIs are not proportional to exposure duration due to metabolic and other elimination processes which occur concurrently with the exposure. First-order clearance models provide an excellent fit to blood and urine data, and these models can be used to estimate exposures and doses. However, clearance rate estimates depend on the BEI,

e.g., urine data provides a 2.23 hour half-life, considerably longer than that estimated using blood data (1.56 hours) or breath data. Concentrations in blood did not peak during or at the termination of exposure, but lagged some 15 to 30 minutes, and concentrations in urine were further delayed. While breath sampling may be the most convenient and practical of the BEIs investigated, breath concentrations reflect alveolar air in equilibrium with blood only if subjects are in a methanol-free environment for 30 minutes or more after the exposure. At earlier times, breath concentrations reflect airway desorption and diffusion processes with relatively slow clearance rates. Based on multicompartment models, the desorption processes have half-lives between 0.6 and 5.0 minutes. These observations have a direct impact on strategies for sampling and interpreting results of breath, blood and urine specimens of workers exposed to methanol.

Publications which have resulted from the project entitled "Biological Monitoring of Methanol Exposure" (5 R01 OH03024-02)

1. Franzblau A, Batterman S, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. Breath Monitoring of Inhalation and Dermal Methanol Exposure. Applied Occup Environ Hyg. 1995;10(10):833-839.

2. Batterman SA, Xiao H, Franzblau A. Blood and urine bioindicators for methanol exposure: Effect of chilled and frozen sample storage. Applied Occup Environ Hyg. 1996;11(1):25-29.

3. Batterman SA, Franzblau A, Zhou N. Airborne emissions at skin surfaces: A potential biological exposure index. Int J Occup & Environ Health. 1996;68(4):268-274.

4. Franzblau A, Batterman SA, Zhou N, Stepien CJ, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. Evaluation of methanol and formate in urine as biological exposure indices of methanol exposure. Applied Occup Environ Hyg. (in press).

5. Batterman SA, Franzblau A. Time Resolved Absorption and Permeation Rates of Methanol in Human Volunteers. Work in progress (Submitted for publication - only abstract included).

6. Batterman SA, Franzblau A, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. Breath, Urine and Blood Measurements as Biological Exposure Indices of Short-term Inhalational Exposures to Methanol. Work in progress (submitted for publication - only abstract included).