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Use of Urinary Formic Acid as a Biologic Exposure Index of Methanol Exposure

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Interest has developed in the use of urinary formic acid as a biologic exposure index of occupational methanol exposure. Four human subjects were exposed to approximately 200 ppm of methanol in a test chamber for 6 hours. Ambient air in the chamber was monitored for methanol, while urine was monitored for formic acid. Urine specimens were collected immediately before, immediately after, and 16 hours following cessation of exposure to methanol. Mean urinary formic acid was increased from baseline at the end of the exposure session (though not significantly), but had returned to baseline in samples collected 16 hours following cessation of exposure. These results suggest that measurement of urinary formic acid in specimens collected 16 hours following cessation of exposure to methanol may not be an appropriate approach to biologically assess methanol exposure. Franzblau, A.; Levine, S.P.; Schreck, R.M.; D'Arcy, J.B.; Qu, Q.S.: Use of Urinary Formic Acid as a Biologic Exposure Index of Methanol Exposure. Appl. Occup. Environ. Hyg. 7(7):467-471; 1992.

Introduction

Methanol is widely used in industrial applications, primarily as a chemical intermediate. Such use has limited potential for large-scale human exposure because the processes involved are usually closed and the number of workers is relatively small.[1] The opportunity for human exposure may increase substantially in the future if methanol is selected as an alternative automotive fuel.[1-3] Acute toxic effects of methanol exposure, primarily via ingestion, are well described.[4] However, human health effects of chronic, low level exposure to methanol are largely unknown. There have been no human epidemiologic studies focused on the effects of chronic low level methanol exposure, and results of animal studies are difficult to extrapolate to humans because of significant differences in the metabolism and toxicity of methanol in most animal models.[5-7]

An essential step in the design and conduct of an investigation of health effects of methanol is to measure exposure. Exposure to methanol can occur via inhalation, ingestion, and transcutaneous absorption,[8] so availability of a biologic exposure index (BEI) would be valuable because monitoring of methanol in air may underestimate the absorbed dose. Attention has focused on various approaches: methanol in expired air,[8-12] methanol in blood,[9-14] methanol in urine,[8,11,13-15] formic acid in blood,[10,13] and formic acid in urine.[10,13-15] The measurement of formic acid in urine collected before the shift at the end of a typical workweek (approximately 16 hours following last exposure) has been proposed as a method of biologically assessing methanol exposure.[15,16]

This article presents the results of a study of controlled human methanol exposure. Formic acid was measured in urine specimens collected before, immediately after, and 16 hours following cessation of exposure to methanol. Our results fail to confirm results of others[15] concerning the utility of monitoring formic acid in urine specimens collected 16 hours following cessation of exposure to methanol, and suggest that additional work may be necessary before promulgation of a standard BEI for methanol that utilizes urinary formic acid in this manner.

Methods

Inhalation Chamber

Human exposures to methanol vapor were conducted in a $3.7 \times 3.7 \times 2.7$ m^3 stainless steel Rochester-type exposure chamber with 45° tetrahedral top and bottom cones, a suspended floor and ceiling, a rest room, and an airlock for entry and exit. The chamber had a total volume of 47 m^3, and a ventilation rate of 14.2 m^3/min (18 chamber volumes/hour) using high efficiency particulate air filtered, purified, and dehumidified air from the building Inhalation Facility Pure Air System. Temperature was controlled at 22 ± 2°C and relative humidity was maintained at 50 ± 5 percent by rehumidification of the inlet air.

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Vaporization and delivery of methanol vapors to the inhalation chamber took place in two steps. The methanol was pumped from a reservoir into the 80°C vaporization section of the generator by an explosion-proof metering pump. The vaporizer had a continuous flow of 200 L/min of filtered, compressed air with a methanol concentration of approximately 15,000 ppm, which is less than the explosive range of 67,000–360,000 ppm. The diluted methanol vapor was metered into the inhalation chamber air flow through a mass flow controller, and the vapors further diluted to achieve the desired 200 ppm concentration.

Exposure Chamber Air Monitoring

The concentration of methanol in the chamber was monitored using a miniature infrared analyzer, MIRAN 1-A, equipped with a closed-loop calibrator. The MIRAN was set at an indicated wavelength of 9.55 μm (1047 cm^{-1}) using an absorbance scale of 1.0 and a path length of 17.25 m. The output was recorded continuously using a Rustrak Ranger data logger (Gulton Co., E. Greenwich, Rhode Island). Calibration was performed using direct injection of methanol liquid into the calibration loop.

Urine Sampling

Urine specimens were collected from each subject immediately before exposure, immediately after exposure, and 16 hours after exposure. Subjects were instructed to refrain from consumption of diet beverages and alcoholic beverages beginning on the evening prior to the day of the experiment until after collection of the final urine specimen. The specimens were analyzed for formic acid, a primary metabolite of methanol, using head space gas chromatography.[17] Specimens were also assayed for creatinine and specific gravity.

Quality Control

Sample batches taken for urinary formic acid were accompanied by one replicate and one spiked sample. These quality controls were indistinguishable from unaltered biologic specimens and were shipped and processed with the sample batches.

Human Subjects

Four adult male subjects were exposed to methanol vapor for 6 hours. None had known occupational exposure to methanol, formic acid, or formaldehyde. All subjects provided written informed consent. The consent form and research protocol had been approved by the Human Subjects Review Committees of the University of Michigan School of Public Health and the General Motors Research Laboratories.

Results

The methanol concentration in the test chamber air was maintained between 192 and 204 ppm (mean 200.3 ppm) for the duration of the 6-hour exposure session, as assessed via continuous monitoring with the MIRAN. Once having entered the test chamber, subjects did not leave until the 6-hour exposure session was completed. This exposure is equivalent to 150 ppm on an 8-hour time-weighted average (TWA) basis.

The results of measurement of formic acid in urine are listed in Table I. All results are displayed in three ways: the concentration of formic acid in urine, the concentration of formic acid in urine corrected for concentration of creatinine, and the concentration of formic acid in urine corrected for specific gravity of urine. Regardless of the method of correction, the pattern is similar: mean urine formic acid concentration was slightly increased immediately following cessation of exposure when compared to preexposure levels, but had returned to baseline values when measured in urine specimens collected 16 hours following cessation of exposure. None of the changes is significant (repeated measures analysis of variance, $p > 0.05$). The limit of detection of formic acid was less than 1 μg/ml.

Spiked samples showed 90.4 percent recovery for formic acid. Replicate analyses of selected samples were within ±1 percent for formic acid in urine.

Discussion

We have performed a human exposure chamber experiment in which subjects were exposed to a known and well-controlled concentration of methanol in ambient air. Biologic monitoring was achieved by measurement of formic acid in urine.

Accuracy of the concentration of methanol in ambient air within the chamber was assessed by use of the MIRAN. In this case, the ideal application of the MIRAN existed: constant relative humidity, constant concentration of methanol, chamber air that had been prescrubbed to remove all gases and vapors that could interfere with the exposure or analysis experiment, repeated, direct calibration of the system, and a highly trained operator (one of the authors: D'Arcy). Therefore, it is reasonable to believe that the results are accurate despite previous tests that show that the MIRAN results may not be accurate under less ideal conditions.[18]

There have been a number of studies that have investigated measurement of formic acid in urine as a biologic index of methanol exposure.[10,13–15,19] However, the specific focus of the present investigation was to assess the utility of measuring formic acid in urine collected 16 hours following

TABLE I. Results of Urinary Formic Acid Measurements*

Urinary Correction Factors	Preexposure	Postexposure	16 Hours Postexposure
Formic acid (mg/L)	14.5 (6.03)	18.5 (9.26)	14.5 (6.03)
Formic acid (mg/g creatinine)	13.4 (1.96)	18.7 (2.84)	13.8 (3.78)
Formic acid (mg/g specific gravity)	14.5 (5.98)	18.5 (9.19)	14.4 (5.95)

*All entries are the mean (± standard deviation) of four subjects.

cessation of exposure as a BEI of methanol exposure. Only one previously published investigation has addressed this issue,[15] and it is the sole basis of the formic acid BEI for methanol promulgated by the American Conference of Governmental Industrial Hygienists (ACGIH).[16] Therefore, the comments that follow pertain mostly to the previous study, and to the reasons why its results may differ from those of the present investigation.

The urine formic acid results of the present study are similar to those of Liesivuori and Savolainen[15] in some respects. Their mean formic acid concentration in morning urine obtained from unexposed subjects was 15.1 mg/g of creatinine, which is similar to the mean preexposure urine formic acid concentration found in our subjects (13.4 mg/g of creatinine). However, they found the concentration of formic acid in urine collected on Friday morning (16 hours postexposure) from exposed subjects ranged from 26 to 98 mg/g creatinine. Our results ranged from 9.7 to 17.7 mg/g (mean 13.8 mg/g of creatinine; see Table I). The mean and range of urine formic acid concentrations measured in specimens collected immediately postshift (Thursday afternoon) from methanol-exposed workers is not reported by Liesivuori and Savolainen, although such specimens were collected and analyzed.

A number of differences in study design may have contributed to the disparity between the results of the present study and those of Liesivuori and Savolainen. They studied workers with occupational exposure to concentrations of methanol in air that may have varied throughout the day, although the 8-hour TWA exposure to methanol ranged from 40 to 160 ppm; the latter result is similar to the 150-ppm (8-hour TWA) achieved in the current experiment. Some of their subjects may have had cutaneous exposure to methanol that contributed to the overall absorbed dose. These factors would most likely have reduced Liesivuori and Savolainen's ability to detect a significant correlation between methanol in air and formic acid in urine. Nevertheless, the correlation of TWA methanol concentration in air with urine formic acid concentration measured 16 hours after exposure was found to be high ($r = 0.81$).[15]

Another factor that may have contributed to differences between the two studies is work load. Previous work indicates that the lung retention fraction of methanol does not vary with ventilation rate.[11] Thus the absorbed dose of methanol would be proportional to ventilation rate, which is usually assumed to be proportional to work load. The ACGIH acknowledges that pulmonary absorption of airborne methanol is dependent on "activity of the worker."[16] In their study Liesivuori and Savolainen do not describe the activity level of their 14 subjects who had occupational exposure to methanol; it may have been high, low, or mixed, and there is no way of knowing how the activity level of subjects in their study compared to the activity level of our subjects. Therefore, it is not possible to assess quantitatively or qualitatively how differing work loads may have contributed to the disparate results, although work load may have been an important factor.

In addition, their subjects were exposed for an entire work shift, which presumably was longer than 6 hours. Most significantly, their subjects had chronic occupational exposure to methanol, and urine specimens were collected at the end of a workweek, presumably when there might be maximal buildup of formic acid in urine as a result of exposure during the preceding work days. No data from this study are presented to support the assumption that there is buildup of formic acid in urine over the course of a week of occupational methanol exposure. However, two studies[20,21] are cited that appear to provide indirect support to the concept that the formic acid concentration in urine increases for many hours following cessation of exposure to methanol. Liesivuori and Savolainen base this approach on a study of formic acid elimination in rabbits,[20] and a previous study of farmers with formic acid exposure.[21] Due to the species variation in methanol and formate metabolism mentioned previously,[5–7] it is not clear how results in rabbits relate to humans.

In a study of farmers occupationally exposed to formic acid it was found that the urinary formic acid concentration continued to increase up to 15 hours following cessation of exposure among exposed subjects.[21] Urine samples were not collected beyond 15 hours. These results do not permit estimation of the half-life of urinary formic acid following methanol exposure. Nor do they suggest an optimal strategy for sampling of urine for formic acid following occupational methanol exposure. Two additional human studies are cited to support these findings; these are discussed below.

As in many other reports of acute methanol intoxication, McMartin *et al.*[22] described the elimination of methanol and formic acid from blood and urine in two methanol-intoxicated patients. Their patients were treated with ethanol, sodium bicarbonate, and hemodialysis. Unfortunately, conclusions about the kinetics of elimination of formic acid in urine are confounded by the effects of treatment; therefore these results can provide no insight into the present discussion.

Another study examined formate in blood "from 11 untreated methanol-poisoned subjects."[23] Although the subjects in that report were not treated medically prior to obtaining blood samples, 8 of 11 had measurable levels of ethanol in their blood, indicating that most, if not all, had ingested ethanol during or after methanol ingestion and prior to hospitalization. Also, the precise time between ingestion of methanol and blood sampling is not given, and formic acid in urine was not measured. Thus that study provides little information that bears on the elimination half-life of formic acid in urine or blood.

The findings in the present investigation and those of Liesivuori and Savolainen are closely dependent on an understanding of the elimination kinetics of methanol and formic acid in humans. The primary metabolic degradation products of methanol are, in order, formaldehyde, formic acid (formate), and carbon dioxide.[24] One report describes the half-lives of methanol, formaldehyde, and formate in

the blood of humans at relevant occupational exposure levels as approximately 3 hours, 2 minutes, and 45 minutes, respectively.[24] These numbers would imply that, with occupational exposure to methanol, the overall half-life of the clearance of formate from blood is probably less than 4 hours. In addition, it is worth noting that well over 90 percent of absorbed methanol is excreted as carbon dioxide and very little is excreted unchanged as methanol (in breath or urine) or as formate (in urine).[24] Thus, collection of urine 16 hours following cessation of exposure to methanol would involve the elapse of approximately four half-lives. On a theoretical basis one would not expect a significant accumulation of formate in urine following the elapse of 16 hours between last exposure to methanol and collection of a urine specimen.

A second report suggests that the elimination half-life of formic acid in urine is on the order of 2.25 hours, although no data are presented to support this estimate.[13] According to this estimate, elapse of 16 hours following cessation of exposure to methanol would represent over seven half-lives, making it almost impossible to detect any significant increase in formic acid in such postexposure urine specimens.

In general, establishment of a BEI for methanol is complicated by a number of factors. Methanol and formic acid are normal trace constituents of body fluids,[25,26] so biologic monitoring has the added burden of distinguishing a normal level from an elevated level — not just identifying the presence of a compound that is totally foreign. Methanol is contained in a variety of foods that are widely consumed in the diet: fruits and fruit juices can contain minute quantities of methanol, and alcoholic beverages contain trace amounts of methanol in addition to ethanol.[27] Some food additives are metabolized to form methanol *in vivo*, such as the artificial sweetner aspartame, and therefore add to the overall body burden.[28–30] Consumption of beverages containing ethanol, aside from the trace quantities of methanol in these beverages, drastically alters the metabolism of methanol because the same enzyme systems are responsible for breakdown of both alcohols.[27] In fact, the concept of interference in metabolism is one of the central components of the treatment of acute methanol intoxication.[22,31] Another factor that complicates the promulgation of uniform biologic exposure indices for methanol is the allelic diversity of alcohol dehydrogenase and aldehyde dehydrogenase, the two critical enzyme systems that serve to metabolize methanol and ethanol.[32] The metabolic rate constants of allelic variants differ substantially.[32] These genetic variants are not rare in some populations,[32] and there is substantial association of genotype with racial types.[33]

Conclusions

In summary, there exists only one other published study that focuses on measurement of formic acid in urine collected 16 hours following cessation of methanol exposure,[15] and the results of that study differ from those in the present investigation in a number of critical respects. Important differences in study design that may have contributed to these disparate results include chronicity of methanol exposure of subjects, duration of work shift with methanol exposure, the possibility of dermal exposure to methanol, fluctuations of airborne methanol concentration during exposure (work) periods, and the impact of work rate or pulmonary ventilation on absorbed dose of methanol.

Our results suggest that the current BEI for methanol based on measurement of formic acid in urine obtained 16 hours following cessation of exposure may be premature, and that additional investigations are required.

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