Am. Ind. Hyg. Assoc. J. 45(1):19-23 (1984)

Monitoring of Formic Acid in Urine of Humans Exposed to Low Levels of Formaldehyde

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This study documented the formaldehyde exposures of a group of veterinary medicine students. It also investigated the feasibility of biologically monitoring the exposures. The biological monitoring was based on the fact that the formaldehyde is metabolized in the body to formic acid, and may then be excreted in the urine. Therefore, exposures to formaldehyde could theoretically create a shift in the formic acid levels in the urine. Normal baseline levels of urinary formic acid were first established for each subject. The baselines of most students were quite variable. Very few exhibited a "tight variability" in their baseline. Next, three sets of pre- and post-exposure urine samples were taken. A series of paired t-tests were run on these "pre" and "post" sets. The results indicated that no significant formic acid shift was seen. A subset of the samples was "corrected" for specific gravity. However, this adjustment did not have an effect upon the relative formic acid levels. In addition, no significant formic acid shift was seen in the adjusted group. Exposure levels of the students were less than 0.5 ppm of formaldehyde. Therefore, the main conclusion of the study was that biological monitoring of formaldehyde exposures (via formic acid shifts) at these low levels was not a feasible technique.

Introduction

Formaldehyde is a widely utilized compound, with applications ranging from the production of resins to the treatment of textiles. Because the use of formaldehyde spans numerous industries, many workers are potentially exposed. Exposure to low levels of formaldehyde raises complaints of eye and upper respiratory tract irritation. Such complaints, along with the recent implications of human carcinogenicity, indicate that close monitoring of formaldehyde exposures is a necessity. The development and use of biological monitoring would assist in the proper assessment of formaldehyde exposures.

This study had two objectives. First, it documented the formaldehyde exposures of a group of veterinary medicine students. Second, the study attempted to evaluate two methods of biological monitoring for formaldehyde exposures. One method of biological monitoring was based on possible urinary formic acid shift due to exposure, while the other was based on possible pulmonary function acid shifts due to exposure.

This portion of the study dealt with formic acid shifts in the urine. Previous studies have attempted to monitor formaldehyde exposures based on formic acid shifts in the urine.[1] Formaldehyde appears to have a short latency period for excretion. Formaldehyde is a normal metabolite in man and also an important ingredient in the synthesis of essential biochemical substances. Thus, small quantities of formaldehyde in tissues are not toxic.[2] However, when formaldehyde levels build up within the body, the body must metabolize the excess formaldehyde. The main reaction is the initial oxidation of the formaldehyde to formic acid in the liver and in the erythrocytes.[3] This formic acid may then take one of three pathways: further oxidation to CO_2 and H_2O; elimination in the urine as a sodium salt; or entrance into the metabolic one-carbon pool.

Formic acid is naturally found in the body. These natural sources of formic acid are from the metabolism of certain proteins and sugars. The amino acid glycine appears to be the main contributor. Excessive amounts of carbohydrates and protein may increase formic acid excretion levels while starvation will cause a decrease in formic acid levels.[4] It has been reported that humans average about 17 mg of formic acid per liter of urine.[5]

Study Design

The research was based on the formaldehyde exposures of the 1981 Freshman Veterinary Medicine Class at Colorado State University. All the freshmen were required to enroll in VM 602, Functional Anatomy I. In the laboratory, the students worked extensively with animals preserved in formalin, as well as fresh animals and specimens.

The 1981 class consisted of 139 students with males and females equally represented. All students were introduced to the research protocol and objectives of this study at the beginning of the fall semester. At this time, each student was issued a short form explaining the requirements of participation in the study. The form also contained a section on which the student could volunteer or decline participation as a study subject. A total of 112 students volunteered. Again, males and females were equally represented.

The 112 volunteers were reduced to 30 males and 30 females by using a table of random numbers. Each of the 60 subjects chosen by random selection was contacted by telephone and asked a series of questions. The purpose of this verbal interview was twofold. First, it was important to

determine if the subject was still willing to participate even though the research would require additional time on their behalf. Second, the oral questionnaire was designed to identify confounding variables that could affect the outcome of biological monitoring. These variables included smoking history, use of certain medications, eating and drinking habits, formaldehyde exposure other than in class, and the presence of illness or disease. Each verbal response was recorded on a standardized form. Based on the information on the questionnaires, 36 students (18 males, 18 females) were chosen as the study population. Students that smoked or used antihistamines or were additionally exposed to formaldehyde outside of class, were all eliminated from the study population, as any of these factors might influence their response to their formaldehyde exposure in the anatomy laboratory. After the study began, one female withdrew from school and hence dropped out of the study, leaving a total of 17 females.

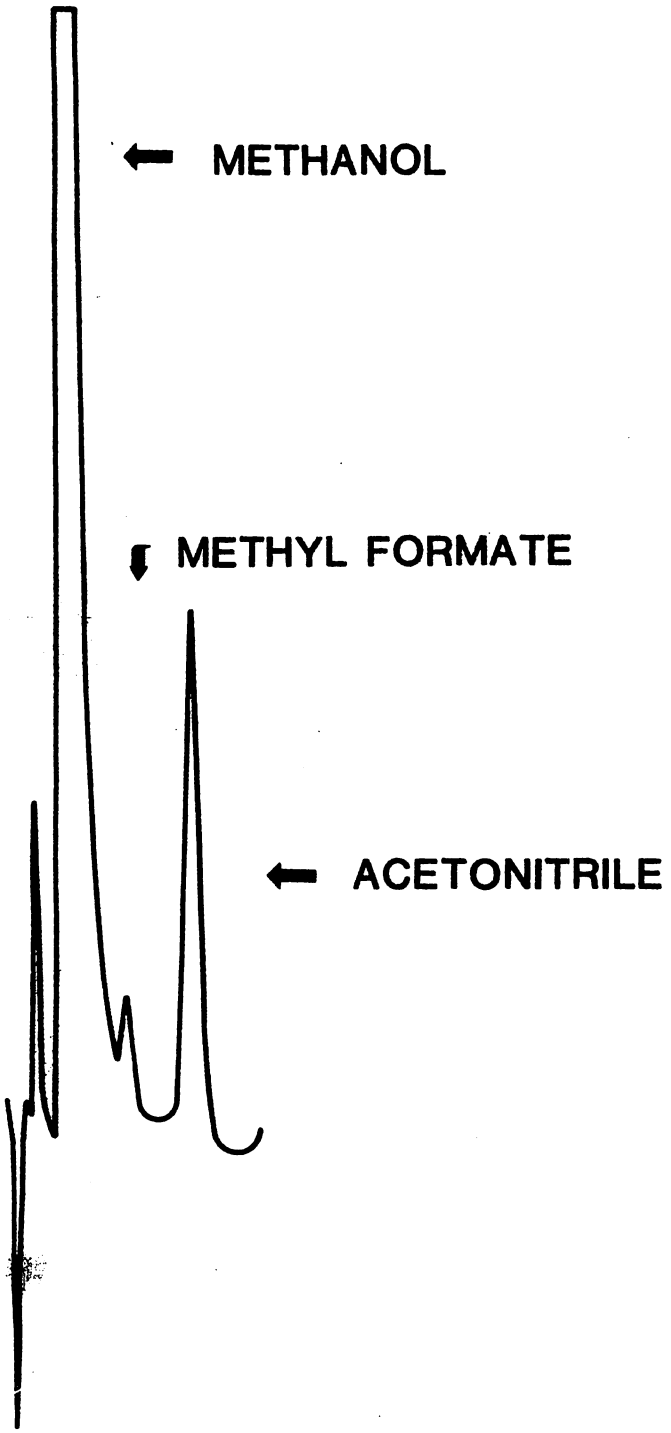

Figure 1 — Chromatogram of a urine sample.

Once the study population was selected, both pulmonary function and urinary formic acid baselines were determined for each subject. All subjects were given a minimum of three pulmonary function tests per day on three different days, for a total of nine tests. Three urine samples (taken one each morning for three consecutive days) were taken from each subject and analyzed for formic acid. The purpose of these baselines was to establish their normal baseline pulmonary function values and normal formic acid levels in their urine.

Documentation of formaldehyde exposure over a three-week period in the middle of the semester began when laboratory work involved the use of animals preserved in a formalin solution containing approximately 40% formaldehyde. Formaldehyde exposure documentation, pulmonary function tests and urine samples were obtained for each subject once a week for three consecutive weeks. Exposure documentation was accomplished using 3M Formaldehyde Monitors. On the morning of a sampling day before exposure in the laboratory, each student gave a urine sample, performed a series of pulmonary function maneuvers, and answered a series of questions designed to further identify confounding variables and the subject's present state of health. Before entering the laboratory, a 3M monitor was placed on the lapel of each student's coat, to be worn while in the laboratory. After the class, approximately two hours after the pre-exposure measurements, each subject turned in the formaldehyde monitor, performed another series of pulmonary function tests and reported any symptoms or signs they were experiencing that had not been occurring prior to the laboratory class. Within two hours after class, each student gave a final post-exposure urine sample.

Methods and Materials

The analytical technique used was adapted from a technique developed by Abolin, *et al.*[6] The technique for formic acid analysis in urine was based on the conversion of the formic acid to methyl formate in the gaseous state. The methyl formate could be detected, then, with a flame ionization detector on the gas chromatograph (GC). Figure 1 is a chromatogram of a urine sample, and shows three of the components of a urine sample, as detected with flame ionization chromatography.

A set of standards was run each day of analysis. These standards were used to quantify the amount of formic acid found in each sample. A peak height ratio of methyl formate to acetonitrile (an internal standard) was calculated for each sample and this ratio inserted into a regression line which had been calculated from the standards. The resulting value was the amount of formic acid in a sample.

The proper quantification of formic acid in the urine samples depended on correctly identifying the peaks seen on

the GC chromatogram. Therefore, it was necessary to confirm that the analytical technique did produce the expected peaks at the expected place on the chromatogram. This confirmation was accomplished with Gas Chromatography-Mass Spectrometry (GC-MS). Figure 2 is the methyl formate peak from a sample and its identification by mass spectrum as methyl formate.

The urine specific gravity was determined for about 25% of the samples using a refractometer, the specific gravity being read from the scale on the refractometer. The average urine specific gravity for white Americans is 1.024.[7] Therefore, the formic acid level in each of the samples (in which the specific gravity was determined) was "corrected." This "corrected" formic acid level would correspond to the amount of formic acid in that sample if the specific gravity was 1.024. This subset was statistically analyzed in order to determine whether specific gravity adjustment was needed to quantitate properly the formic acid levels.

Results and Discussion

The mean urinary formic acid baseline for the 35 subjects was 12.47 mg/L. There was great variability in formic acid levels not only within subjects, but also between subjects. Only about 33% of the subjects had three baselines that varied by <5 mg/L. Individual mean formic acid baselines ranged from 2.43 mg/L to 28.38 mg/L.

TABLE I
Summary of Means, Ranges, and FA Changes

	Week 1	Week 2	Week 3
Mean FA Level (mg/L)			
Pre	13.16	11.59	13.96
Post	13.55	11.70	16.40
Range of FA Changes (mg/L)	-7.43→ 18.65	-9.73→ 14.82	-38.74→ 28.29
Mean FA Change (mg/L)	0.39	0.10	2.44
Exposure Range (ppm)	.04→ .33	.02→ .36	.00→ .23
Mean Exposure (ppm)	.11	.11	.04

Table I summarizes the formic acid levels and changes, and exposure levels for each of the three weeks. The formic acid (FA) change, or the shift, was the "post" formic acid (FA) value minus the "pre" formic acid value. The variability of the FA change was large. The negative numbers indicated that the formic acid level was lower at post-exposure than it was at pre-exposure. Week 3 had a very negative FA change of -38.74. This was due to one subject having a very

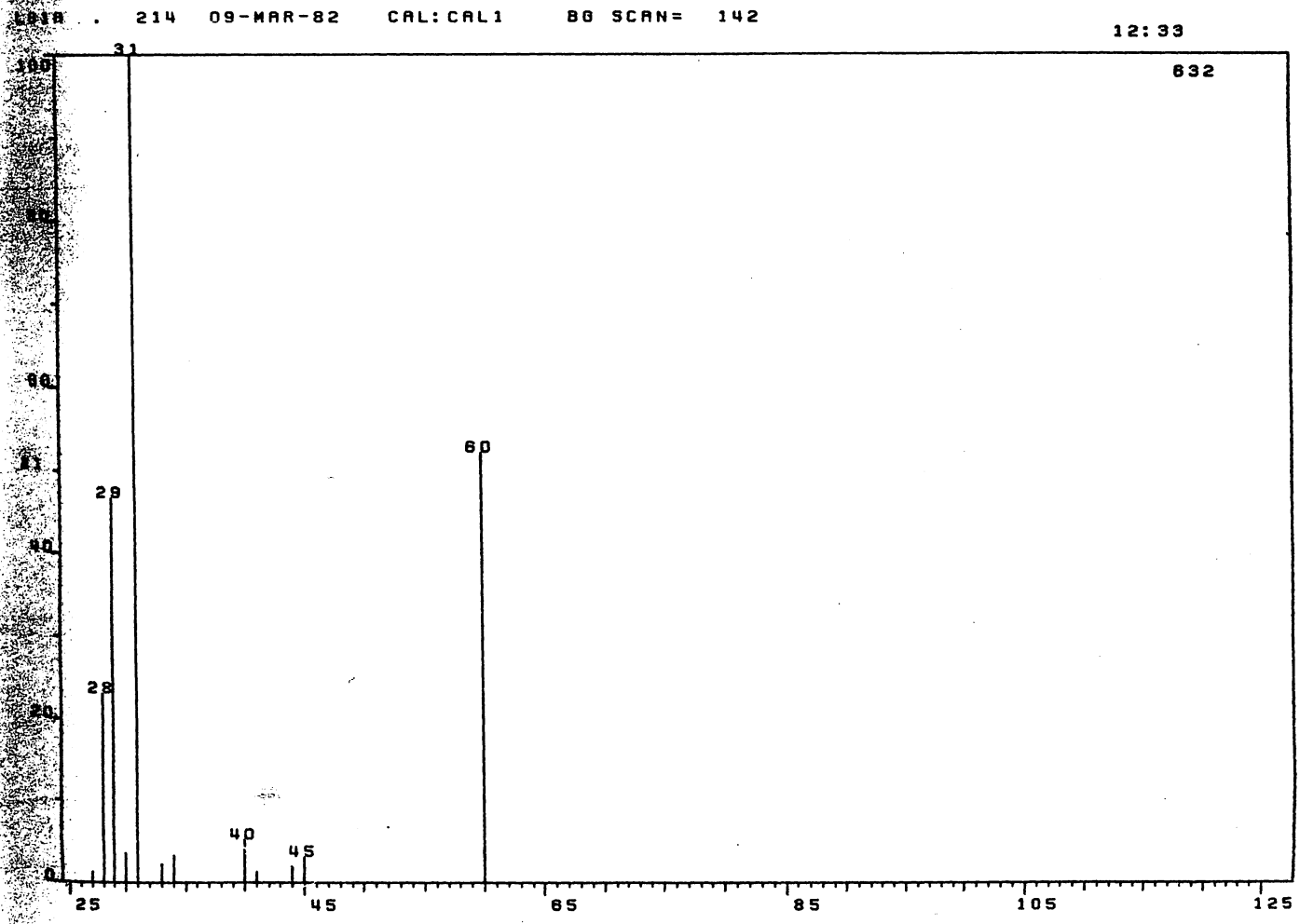

Figure 2 — Methyl formate confirmation by GC-MS.

high formic acid level for her "pre" FA. This FA value was completely out of the range of her baselines, or any other readings from the study. Why she had this very high FA value was unknown.

The mean "pre" FA value for Week 1 was 13.16 mg/L, while the mean "post" FA value for Week 1 was 13.55 mg/L. This indicated that no significant FA change occurred for Week 1, although further statistical analysis was necessary. Therefore, a paired t-test was run on the FA values for Week 1. The t = -.3560, with 32 degrees of freedom (d.f.), was not significant (α = 0.05). This meant that there was no real difference between "pre" and "post" FA values.

As was seen in Week 1, the two means for Week 2 were very similar, indicating no significant FA change that week. A paired t-test was run on the FA values to determine any statistical significance. For Week 2, t = .3517 (with 31 d.f.). Again, this was not significant (α = 0.05).

The difference between "pre" and "post" FA levels of Week 3 was greater than the difference seen in Week 1 or Week 2. This indicated that possibly a shift had occurred. In order to determine if an actual shift occurred, a paired t-test was run resulting in t = 1.3262 (with 32 d.f.). Again, like Week 1 and Week 2, the t-value was not significant at α = 0.05.

The passive monitor readings for all three weeks indicated that the formaldehyde levels were well below the threshold limit value (TLV®) of 2 ppm (ceiling). Week 3 had almost 50% of the subjects registering a "zero" exposure. The reason that exposure levels were so low for Week 3 was that many of the students dissected fresh animals that week. While some students did work on their embalmed dogs, many dissected fresh dogs and horses.

There never appeared to be any relation between exposure levels (as determined by the passive monitors) and the presence or lack of FAC. Some students had a high FA change, but this never consistently correlated to one of the higher exposure levels.

In addition to documenting formic acid levels and exposure levels, the study also documented any subjective symptoms reported by the students. Each time the students were monitored, they were asked if they noticed any problems (*i.e.*, sore throat, tearing of the eyes) during their work in the laboratory. Some of the symptoms reported were three cases of headache, three cases of burning sensation of the eyes, one case of sneezing, one case of "scratchy" throat, and one case of rash on the forehead (from touching his forehead with his formalin contaminated gloved hands). There did not appear to be a relation between the symptoms and/or their severity and the exposures recorded by the passive monitors. The majority of the students reported no problems.

Twenty-seven sets of pre- and post-exposure urine samples (25% of the total 108 sets) had their specific gravity determined with a refractometer. The mean specific gravity values of all the "pre" urine samples was 1.021, while the mean of all the "post" was 1.024. A paired t-test was run on the "pre" and "post" specific gravity values to determine if there was a significant difference between "pre" and "post." The test gave a t = -1.614 (with 26 d.f.), which was not significant at α = 0.05. A correlation coefficient was calcu lated. The coefficient r, was equal to 0.724. These two tes together meant that there was no significant differen between "pre" and "post" specific gravity. Hence, it w assumed that even if formic acid excretion levels were relat to specific gravity, this relation would be negated by the l of "pre" and "post" specific gravity difference. In ot words, if the specific gravity was low (or high) for the "p urine sample, it was probably low (or high) for the "po urine sample. Therefore, the relative difference in for acid levels would not be affected. This did *not* mean spec gravity did not affect formic acid levels, only that the de mination of specific gravity was not necessary to deter proper formic acid shifts.

Table II lists the "uncorrected" and the "corrected" for acid values for the subset. Paired t-tests were run on b sets of values. Both t-tests found no statistically signifi difference between "pre" and "post" formic acid values.

Multiple analysis of variance tests were run on the full of data. The analysis was carried out considering all co nations of the factors. The results indicated that no stat cally significant relations were found between any o factors, such as age, sex, week, exposure values, and post formic acid values.

TABLE II
Corrected Formic Acid Values for Subset

	Formic Acid Levels (mg/L)			
	Pre-Exposure		Post-Exposure	
I.D. #	Observed	Adjusted	Observed	Adjust
Week #1				
1	4.91	19.64	23.56	37.7
2	25.09	18.82	28.20	19.3
4	5.40	9.26	4.96	10.8
6	9.37	11.24	3.56	6.1
14	20.66	24.79	17.03	27.2
20	10.54	7.66	4.19	2.7
25	13.29	11.39	14.23	14.8
34	21.17	14.52	13.74	10.3
36	15.63	17.86	18.33	16.2
Week #2				
1	4.01	16.04	18.83	30.1
2	2.43	9.72	10.81	13.8
6	1.71	8.21	1.35	2.9
10	10.91	23.80	3.56	17.0
28	24.14	16.09	24.50	21.0
29	20.86	14.72	14.23	9.3
30	6.40	9.04	5.36	12.9
32	22.52	12.28	15.76	11.3
36	27.61	23.66	17.88	15.3
Week #3				
1	10.99	52.75	27.03	36.0
2	9.23	18.46	23.47	20.2
8	83.11	48.65	44.37	26.1
10	1.49	2.98	2.93	7.0
15	3.69	3.69	10.27	8.2
17	1.80	3.32	16.49	13.3
25	19.68	14.76	16.98	11.9
35	5.99	5.75	11.98	11.7
36	7.97	15.94	36.26	25.8

Conclusions

All of the analyses were based upon the paired t-tests. The paired t-test only looked at the group as a whole, to help decide if there was an FA change for the group as a whole. Many individuals did have positive FA changes; a few consistently had large FA changes each week. Because the study group as a whole did not exhibit an FA change, it was difficult to attribute the individual FA change as a result of formaldehyde exposures.

A problem encountered after the study began was that the variability in FA baselines was much higher than expected. This variability made it difficult to determine whether an individual's FA change was from natural fluctuation or from an exposure to formaldehyde. Perhaps it would have been helpful if two baselines had been taken each day, rather than the one each day. One could have been taken in the morning and one at noon, to correspond to the times that pre- and post-exposure samples were taken during the actual exposure period. These two baselines would have shown any fluctuation in FA levels that naturally occurred within the span of several hours in one day.

Data variation for each of the three weeks was not significant. This meant that there was no statistically significant difference between "pre" and "post" FA values; hence, there was no significant shift seen in urinary formic acid excretion levels. In addition, no cumulative shift was indicated from the FA levels seen or by any of the paired t-tests.

Two conclusions were drawn from the study. First, the exposures to the veterinary students were well below the TLV®. Second, at these low levels, biological monitoring of urinary formic acid levels within two hours of a two-hour formaldehyde exposure did not appear to be useful. The variability in urinary formic acid levels masked any small shifts which might have occurred. In other words, this variability, together with the low exposure levels, did not produce any significant urinary formic acid shifts.

Acknowledgements

A special thanks is extended to all who assisted in and contributed to this work, especially Dr. Lee Wilke, Mike Adams, Craig Beck, Mike Gress, Dan Pacheco, Del Sandfort, and Emilie Wheeler.

Appreciation is also due to the veterinary students and their professors, whose help and participation made the study a success.

The research was financed in part by the National Institute of Occupational Safety and Health (NIOSH) training grant number 5T01 0H0 7039-06.

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6 July 1982; Revised 26 August 1983