Lack of Specificity of *trans,trans*-Muconic Acid as a Benzene Biomarker after Ingestion of Sorbic Acid-preserved Foods[1]

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Abstract

The benzene metabolite, *trans,trans*-muconic acid (MA), has been shown to be a sensitive and specific biomarker for ambient benzene exposure levels as low as ~0.5 ppm. However, at lower exposure levels, the use of MA as a benzene biomarker is complicated by the fact that it is also a metabolite of the food preservative, sorbic acid. To better assess the extent of this interference, MA was measured in sequential spot urine samples over a 2-day study period from eight volunteers (four adults and two parent-children pairs) who consumed two sorbic acid-preserved foods. Large increases in MA concentration were seen after ingestion of both foods. Individual peaks ranged as high as 1673.7 ng/ml (705.3 ng/mg creatinine) in adults and 1752.1 ng/mg creatinine (1221.3 ng/ml) in children. Ratios of peak to baseline values varied from 2.5 to 60. The average peak in the seven subjects who showed an increase in MA after ingestion of the first sorbic acid-containing food was 531.1 ng/ml (693.2 ng/mg creatinine). The average in the seven participants who ingested the second food was 1102.1 ng/ml (795.3 ng/mg creatinine). Twenty-four-hour personal air benzene levels were all low (≤5.6 ppb). Substantial variation in MA results were seen in some males related to creatinine adjustment. These data indicate that sorbic acid-preserved foods have the potential to cause substantial interference with MA as a biomarker for both occupational and environmental benzene exposure in populations, such as in the United States, where consumption of preserved foods is common. Development of methods to minimize and/or assess sorbic acid interference will improve MA specificity in such populations.

Introduction

Environmental benzene exposure is ubiquitous because of its presence in gasoline and tobacco smoke (1). Occupational exposure also occurs in a variety of workplaces. Because benzene is a human carcinogen, measurement of exposure biomarkers, which provide information on individual variation in absorption and metabolism and, thus, susceptibility for adverse health effects, is useful in the protection of exposed populations. Urinary phenol has been used as a biological monitoring tool for benzene-exposed workers; however, it lacks specificity for ambient exposures <5 ppm (2). Because benzene exposure limits are now generally much lower than this, phenol is currently useful as a benzene biomarker only after accidental high-level exposures.

As a result, researchers have explored the biomarker potential of several other benzene metabolites as well as the parent compound itself. MA,[3] a straight chain metabolite, has been shown to be a sensitive and specific biomarker for exposures as low as 0.5 ppm. The assay is relatively fast and simple, making it ideal for medical surveillance and epidemiological studies requiring analysis of large numbers of samples. However, the use of MA as a biomarker for low-level benzene exposure is complicated by the fact that, as with phenol, it is not completely specific for benzene either. Sorbic acid and potassium sorbate, which are food preservatives, are also metabolized to MA (3). Studies evaluating this metabolism have found that approximately 0.05–0.5% of ingested sorbic acid tablets is metabolized to MA (4, 5). Sorbic acid-based preservatives are used in several food categories including processed cheese slices and spreads, refrigerated flavored drinks, sweet baked goods, mayonnaise, margarine, and salad dressing.

Results of recent studies assessing the urinary MA response after ingestion of sorbic acid or potassium sorbate in pill form suggest that the specificity of MA as a biomarker for low-level benzene exposure may be limited in populations with substantial consumption of sorbic acid-based preserved foods (4–7). However, the actual extent of this interference is unknown because the amount of sorbic acid in the diet must be estimated. Therefore, we measured MA levels in sequential spot urine samples from volunteers who consumed sorbic acid-containing foods that are common in the United States diet and usually ingested in substantial amounts when consumed. To our knowledge, this is the first report of MA levels obtained after ingestion of actual foods containing sorbic acid-based preservatives and the first to include children.

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[3] The abbreviations used are: MA, *trans,trans*-muconic acid; HC, Hostess cupcakes; HPLC, high-pressure liquid chromatography; LD, limit of detection; oz., ounces; NIOSH, National Institute for Occupational Safety and Health; SunD, Sunny Delight; OSHA, Occupational Safety and Health Administration; NIEHS, National Institute of Environmental Health Sciences.

Materials and Methods

Study Population. Study participants were recruited from the Johns Hopkins University School of Hygiene and Public Health. Exclusionary criteria included chronic illness, medication use, and smoking. These exclusions were designed to reduce potential sources of metabolic differences that could affect biomarker levels and to eliminate main stream tobacco smoke as an environmental source of benzene. Explanations to the adults and children (appropriately age-modified) were provided, and informed consent, approved by the Joint Committee on Clinical Investigation (Johns Hopkins University School of Medicine and the Johns Hopkins Hospital), was obtained from all participants.

Study Design. Adult subjects were studied over a 48-h period, and children were studied for a 24-h period (one child completed the full 48-h study). Specific foods thought to have the highest potential for sorbic acid interference with MA as a benzene biomarker were ingested at lunch on each of the two study days. These items were selected by a review of literature on foods known to contain sorbic acid-based preservatives (8, 9), examination of food labels, and estimation of the amount of such foods usually consumed at one sitting. On the basis of this information, 16 oz. of Sunny Delight was ingested with the noon meal on the first study day (ID 2 drank 12 oz.), and one 3 oz. package of HC (two cupcakes) was consumed on day 2. ID 1 completed a third study day and consumed a processed cheese sandwich. Other food consumption consisted of the participant's normal diet, except for efforts to avoid foods containing sorbic acid-based preservatives. Subjects were advised of foods likely to contain these preservatives and were asked to examine food labels for sorbic acid or potassium sorbate.

Self-administered questionnaires (completed by parents for children) on demographics and sources of benzene and dietary sorbic acid-based preservatives in the preceding 24 h were administered at the beginning of the study. No subject had an occupational source of benzene. Environmental sources of benzene exposure were noted such as environmental tobacco smoke exposure, time spent in an automobile or bus, and pumping gas. Daily logs to record sources and times of benzene exposure and foods ingested in each 24-h time period during the study were completed, as was a daily log of urine collection times.

Inhalation exposure to benzene was evaluated using a modification of NIOSH Method 1500 (10), consisting of 24 h integrated air samples actively pumped onto coconut shell charcoal tubes (SKC Inc., Eighty Four, PA) for five of the six adults studied. Samples were collected at a nominal flow rate of 90 ml/min using a small, low-flow personal pump (model P200; DuPont, Wilmington, DE), resulting in a nominal sample volume of 130 liters in 24 h. Sampling flow rates were checked when sampling was begun and again at the completion of each 24 h sampling period. Duplicate samples were collected each day in four adult subjects; three laboratory blanks were included. After collection, samples tubes were sealed and stored at −20°C until extracted. For the other three subjects, 24-h personal monitoring was conducted using passive vapor sampling (3M OVM badges; 3M, St. Paul, MN).

Twenty ml urine samples were collected from each urine void during the 48-h study period. Samples were frozen after collection and transported to the laboratory in an ice chest daily. Samples were stored at −80°C until analyzed.

Chemicals. MA standards were prepared as described previously using chemical purchased from Aldrich Chemical Co. (Milwaukee, WI; Ref. 11). Sorbic acid was purchased from Sigma Chemical Co. (St. Louis, MO) and prepared in the same manner as the MA standard. All water used was purified by a MilliQ Water System (Millipore Corp., Bedford, MA). Acetone was obtained from Burdick and Jackson (Muskegon, MI). Low-benzene carbon disulfide was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of the highest quality obtainable commercially.

MA Assay. The HPLC-UV assay used was described previously (11). Briefly, a 1-ml urine sample was adjusted to pH 4.5–5.75 and extracted on a PrepSep strong anion exchange cartridge (Fisher Scientific, Fair Lawn, NJ). Twenty μl of the 3-ml eluate were injected. The HPLC column was an Altima C18 5 μm (25 cm × 4.6 mm) analytical column preceded by a 5 μm (7.5 × 4.6 mm) Altima guard cartridge (Alltech Associates, Inc., Deerfield, IL). Chromatography was isocratic in a mobile phase consisting of 0.45% glacial acetic acid, 0.18% 1 M sodium acetate, and 10% methanol at a flow rate of 1 ml/min. The column temperature was maintained at 40°C. Diode array detection was used to assess spectra from all peaks as additional confirmation. Ten % duplicates were performed, including all peaks.

Urinary Creatinine Assay. Creatinine was measured using the Sigma kit (St. Louis, MO). This assay is based on Heinegard and Tiderstrom's (12) modification of the Jaffé colorimetric assay that measures difference in absorbance at 500 nm of the creatinine-picrate chromogen before and after acidification. Absorbance was measured in a Beckman DU-7 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The assay described in the kit was modified to use a third of the suggested sample and reagents. This modification was validated by comparing results on 20 specimens with an automated creatinine assay, using a Boehringer Mannheim/Hitachi 917 Analyzer. A correlation of 0.999 was found between the two assays. The slope was 0.965 (SE, 0.01), and the intercept was −1.09 (SE, 1.55).

Air Benzene Assay. Samples were extracted by removing the activated charcoal from the front and backup stages of the tube. Each stage was extracted for 50 min in 1 ml of a solution of 2:1 acetone:carbon disulfide with sonication in an ice solution (10). The resulting supernatant was filtered using an Acrodisc 4CR PTEF syringe filter (Pall Gelman, Ann Arbor, MI). The filtered extract was transferred to a Hewlett Packard 5980 GC (Hewlett Packard Co., Palo Alto, CA) with autosampler. One μl of sample extract was injected onto a Restek RTX–624, 60 m × 0.25 mm inside diameter, 1.4 μm thickness column and detected by mass spectrometry (HP 5971) using single ion monitoring. Samples were blank corrected and quantified from the response factor given from the internal standard, 1,4-difluorobenzene, and the injection of five standards (0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 μg/ml). Benzene recovery from charcoal tubes was estimated to be 97% (SD of 10%) by the analysis of five tubes spiked with 200 ml of a 5.23 ppm by mole VOC compressed gas in nitrogen mix that included benzene (Scotty IV Mix 606, Plumsteadville, PA), yielding a spike of 3.4 μg of benzene. All results reported have been adjusted for recovery. Ten % of the backup sorbents were extracted and analyzed; no breakthroughs were detected. The badges were extracted and analyzed using the same method as for the SKC charcoal tubes.

Statistical Analysis. Values below the MA limit of detection (16 ng/ml) were set at half that value for data analysis ($n = 9$; 5%) (13). Analyses were performed with Excel (Microsoft Corp., Redmond WA) and Sigmastat (Jandel Corp., San Rafael,

Table 1 Demographics and results of SunD ingestion

ID	Sex	Age	Baseline (ng/ml)		Peak (ng/ml)	Ratio	Baseline (ng/mg creatinine)	Peak (ng/mg creatinine)	Ratio	Peak length (h)
			n	Mean (SD)			Mean (SD)			
1	M	38		NA[a]	NR[b]	NA	NA	NR	NA	NA
2	F	5	1	24.4 (NA)	1221.3	50	34.2 (NA)	1752.1	51.2	5.8
3	M	45	3	43.3 (26.7)	535.1	12.4	28.5 (5.0)	426.5	15	4.3
4	M	10	2	61.2 (4.2)	154.6	2.5	45.7 (4.5)	840.4	18.4	7
5	F	39	3	19.9 (10.2)	446.9	22.5	47.3 (6.7)	1008.8	21.3	7.3
6	M	29	2	39.8 (19.7)	593.7	14.9	26.9 (16.4)	253	9.4	6
7	M	39	2	56.2 (4.6)	505.5	9	49.5 (14.1)	432.4	8.7	7.8
8	M	48	3	16.0 (13.5)	260.7	16.3	27.3 (14.3)	139.2	5.1	9.7

[a] NA, not applicable.
[b] NR, no response.

Table 2 Results of HC ingestion

ID	Baseline (ng/ml)		Peak (ng/ml)	Ratio	Baseline (ng/mg creatinine)	Peak (ng/mg creatinine)	Ratio	Peak length (h)
	n	Mean (SD)			Mean (SD)			
1	4	60.1 (36.1)	674.3	168.6	91.0 (43.8)	667.7	7.3	7.1
3	3	30.6 (24.5)	825.7	275.2	27.4 (12.8)	795.5	29.0	8.1
4	3	58.9 (42.9)	1366.0	455.4	53.7 (35.0)	1514.8	28.2	7.2
5	2	12.8 (6.5)	772.4	386.2	18.8 (1.8)	747.3	39.7	9.9
6	2	74.2 (61.5)	1673.7	836.9	33.8 (18.0)	705.3	20.9	8.9
7	4	96.5 (42.9)	1233.6	308.4	56.9 (24.5)	572.4	10.1	10.0
8	4	54.5 (29.7)	1169.0	292.3	66.0 (29.8)	563.9	8.5	10.7

CA). Sigmaplot (SPSS, Inc., Richmond, CA) was used for graphics.

Results

The eight participants in the study included four adults and two parent-child pairs. Age and gender are displayed in Table 1 along with MA results, with and without creatinine adjustment, from subjects before and after Sunny Delight ingestion. Baseline data include the number, mean and SD of MA levels prior to ingestion. Peak is the largest postingestion MA value obtained. The peak values without creatinine adjustment were sometimes from different urine samples than the peak values with adjustment, depending on urine concentration. All but one subject (ID 1) showed an increase in MA levels over the baseline. (The lack of MA increase in this individual was confirmed by repeating the SunD ingestion. Interestingly, this participant did show an increase after ingestion of two other sources of sorbic acid-based preservatives (cupcakes and processed cheese). ID 8 also had a rather small MA increase after ingestion, although it was still discernable. As shown by the peak:baseline ratio, the increase was generally substantial, ranging from 2.5 to 51. Peak length shows the time that MA in each urine void remained elevated above the baseline. This is a minimum value because urine collections were spaced farther apart at night so the exact time that the baseline was reached cannot be determined. Therefore, SunD ingestion resulted in elevated MA levels for a time period of at least 4.3–9.7 h.

Table 2 depicts the same information before and after cupcake ingestion for the seven participants who completed the second day of the study. Again, peak:baseline ratios were highly variable, ranging from 7.3 to 60.3. Individual SunD and HC ratios were not correlated. The peak length after cupcake ingestion was significantly longer than that for SunD (mean SunD peak length, 6.8; HC, 8.8; $P = 0.04$); this may be related to ingestion of liquid *versus* solid or to the amount of preservative contained in the product.

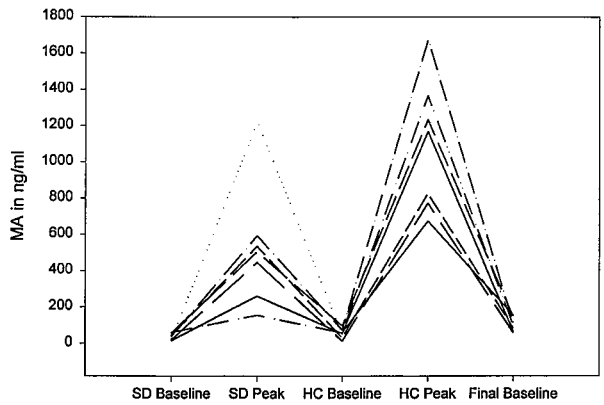

Fig. 1. Baseline and peak MA values, in ng/ml, before and after SunD and HC ingestion.

Fig. 1 is a graphical display of the baseline and peak MA values, in ng/ml, related to the two ingestions; Fig. 2 shows these results after creatinine adjustment. Fig. 3 (ID 5) is a typical result from the study. The *first arrow* indicates the SunD ingestion time; the *second arrow* is the HC ingestion the next day. Figs. 4 and 5 (IDs 2 and 4) display graphs of the children's results. In addition to showing a striking MA response to ingested sorbic acid-based preservatives, these graphs illustrate the importance of adjustment for urine concentration in children after ingestion of a substance in liquid form. The SunD peak

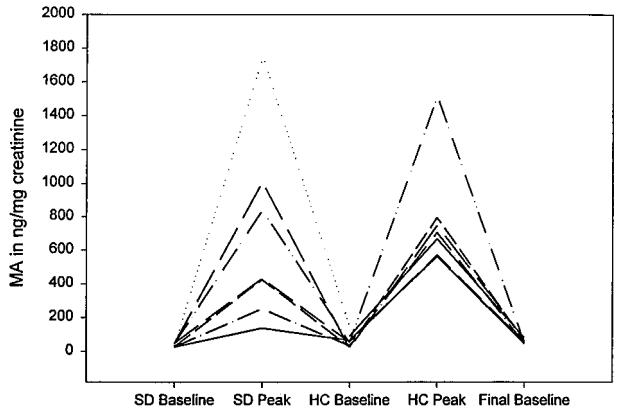

Fig. 2. Baseline and peak MA values, in ng/mg creatinine, before and after SunD and HC ingestion.

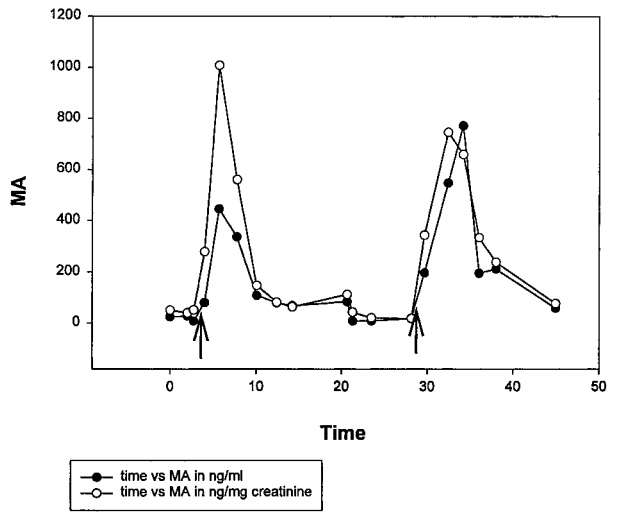

Fig. 3. MA results for ID 5 by time (in hours) from start of study. *First arrow* is time of SunD ingestion; *second arrow* is time of HC ingestion.

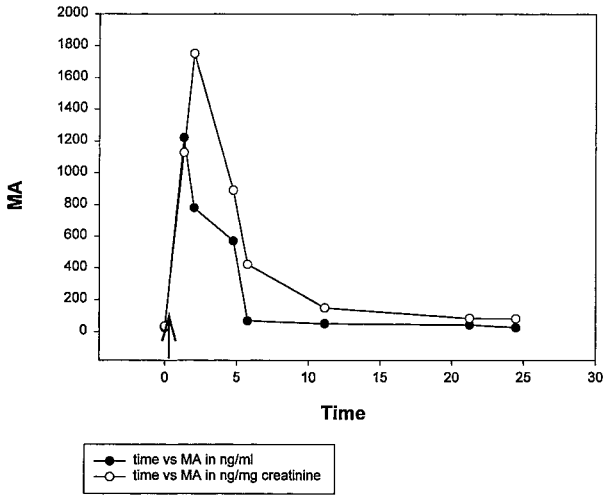

Fig. 4. Results of SunD ingestion in ID 2, showing results in a child and illustrating the need for correction of urine concentration in children.

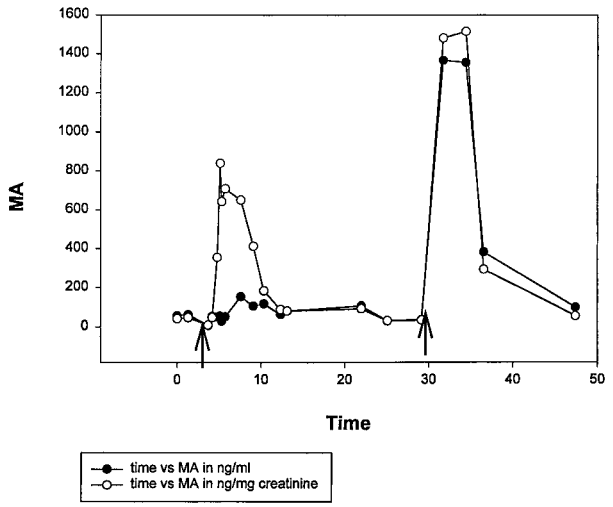

Fig. 5. Results in ID 4, also demonstrating the need for correction of urine concentration in children. The SunD peak is barely discernable without creatinine adjustment.

is barely discernable without creatinine adjustment in Fig. 5. Fig. 6 (IDs 6 and 7) illustrates the opposite problem. When urinary creatinine is relatively high, such as is found in males compared with females, especially in actively exercising males (14), adjustment of urine concentration with creatinine substantially lowers the biomarker result.

In a separate experiment, ID 5 ingested SunD and HC together over a 2-h period. This resulted in a MA peak of 2756.0 ng/ml (3131.8 ng/mg creatinine). Ingestion of a processed cheese sandwich resulted in a MA peak of 576.8 ng/ml (602.4 ng/mg creatinine) in ID 1.

To ensure that the increased MA levels were not attributable to urinary sorbic acid coeluting with MA, a sorbic acid standard was prepared and injected under the HPLC conditions used for MA analysis. No peak was obtained during a 45-min run (MA retention time, ~12 min). When the concentration of methanol was increased from 10 to 50%, a sorbic acid peak with a retention time of 7.7 min was obtained. We also confirmed that exceptionally high environmental benzene exposures were not a factor in our MA results. The 24-h benzene air sampling results ranged from <0.99 (LD of the 3M OVM badges) to 5.6 ppb.

Discussion

MA has been found to be a sensitive and specific biomarker for benzene exposure levels above ~0.5 ppm. However, a study of major industries with benzene exposure found that 60% of employees currently have exposures to air levels ≤0.1 ppm (6). Environmental levels are even lower, with personal exposures averaging 4.7 ppb in the United States Environmental Protection Agency Total Exposure Assessment Methodology studies (1). At these lower exposure levels, the use of MA as a benzene biomarker is complicated by the fact that it is not completely specific because it is also a metabolite of sorbic acid. Recent studies have shown increases in urinary MA after ingestion of pure sorbic acid. The impact of this on MA specificity as a

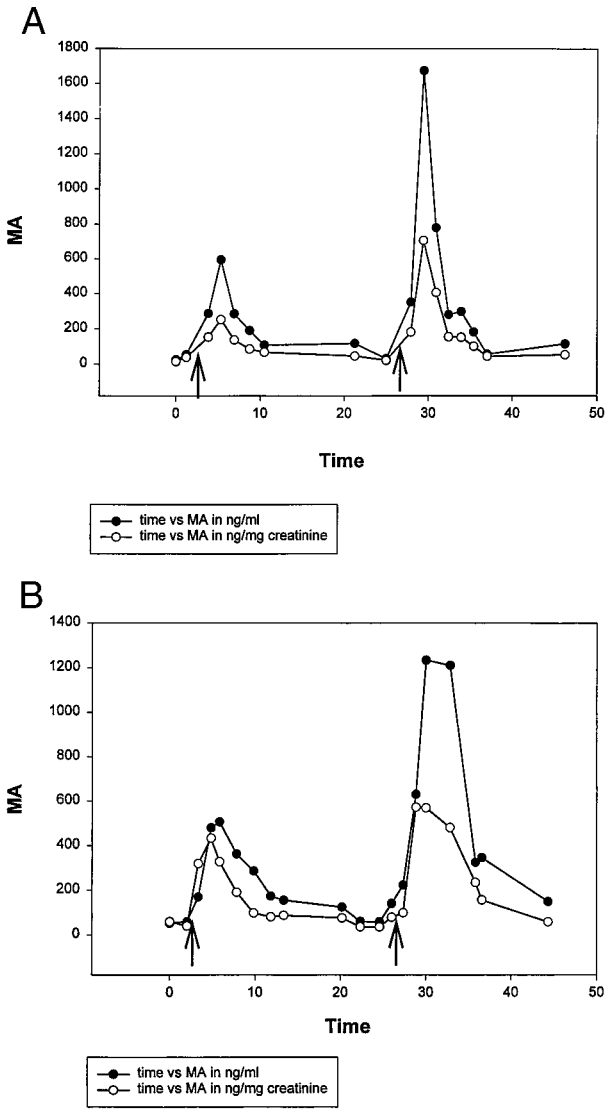

Fig. 6. Results from IDs 6 and 7, which display reduction in biomarker levels after adjustment with relatively high creatinine values. These graphs also illustrate smaller MA increases from SunD compared with HC seen in some subjects.

benzene biomarker has been unclear because the amount of sorbic acid-based preservatives in the diet must be estimated from limited data. Therefore, we measured MA levels in sequential spot urine samples from volunteers who consumed sorbic acid-containing foods. We found that refrigerated flavored drinks and sweet snack foods resulted in the excretion of large amounts of MA in adults and children. This work provides information on the extent of nonspecificity that could occur in occupationally or environmentally exposed subjects in the most common medical surveillance/field conditions that generally involve MA measurement in a spot urine sample at the end of the air monitoring period.

Table 3 displays reported and predicted results from studies that have examined the association of MA with 8 h of air benzene exposures near or below 1 ppm. MA levels are displayed for three specific air benzene levels. One ppm is the OSHA permissible exposure limit (15). 0.5 ppm is the OSHA action limit, which triggers medical surveillance activities, and is the recently reduced American Conference of Governmental Industrial Hygienists' threshold limit value for benzene (16). As noted above, current workplace exposures are reflected by the 0.1 ppm level. In addition to these individual studies, review articles also provide a source of estimated MA values. Ducos and Gaudin (17), using studies from the late 1980s and early 1990s, estimated that an 8-h air benzene exposure of 1 ppm would result in MA levels ranging from 1000 to 2000 ng/ml (500–1500 ng/mg creatinine). Scherer *et al.* (18), based on a large number of studies including several cited in Table 3, estimated that this exposure would result in MA values of 2500 mg/l (1900 ng/mg creatinine).

A wide range of reported and predicted MA values for a given benzene air level is apparent based on Table 3 and the reviews just cited. Smoking, dermal absorption, and interlaboratory differences in assay have been implicated as possible explanatory factors (19). Despite this variation, it is clear, from a comparison of results obtained in Tables 1 and 2 with reported and predicted MA values from actual benzene exposure, that ingestion of foods containing sorbic acid-based preservatives has the potential to decrease MA specificity so significantly that it will confound its use as a benzene biomarker for occupational as well as environmental exposure. The average MA peak after SunD ingestion in the five adults who responded was 468.4 ng/ml (452 ng/mg creatinine). The mean after HC ingestion in six adults was 1058.1 ng/ml (675.3 ng/mg creatinine). These means are greater than any of the predicted values cited in Table 3 for 0.1 ppm benzene exposure, above several values for 0.5 ppm, and even above some predicted values for 1 ppm. The combined ingestion by ID 5 resulted in levels above those predicted for 1 ppm benzene exposure from all of the above cited references, except Kivistö *et al.* (20).

The first study to address the potential effect of sorbic acid on MA specificity as a benzene biomarker found that MA levels in spot urine samples increased from a baseline below the limit of detection (40 ng/ml) to 610 and 660 ng/ml in two volunteers 3–4 h after ingesting a 200-mg dose of sorbic acid (21). These levels decreased rapidly thereafter but did not reach the prior baseline until the next day. Ruppert *et al.* (5) obtained 24-h urine specimens in eight nonsmokers and 2 smokers before, during, and after 2 days of supplementation with 500 mg of sorbic acid/day. They found an 11-fold increase in 24-h MA excretion from a mean of 0.08 to 0.88 mg. Pezzagno and Maestri (6) measured MA in sequential urine voids from two volunteers who ingested 600 and 60 mg of potassium sorbate (447 and 44.7 mg sorbic acid). Compared with the previous 24-h period, they found increases of 21- and 25-fold in the total amount of MA excreted after ingestion of the higher dose and 2- and 3-fold after the lower dose. The peak values were 7070 ng/ml (4842 ng/mg creatinine) and 3414 ng/ml (2492 ng/mg creatinine) after the higher dose and 216 ng/ml (144 ng/mg creatinine) and 120 ng/ml (85 ng/mg creatinine) after the lower dose. These levels represent greater than 90- and 3-fold increases from baseline. A follow-up study measured the increase in 24-h urinary MA levels after ingestion of 600 mg of potassium sorbate in 20 nonsmokers (4). Mean MA excretion increased 23-fold from 64 to 1478.9 μg/24 h.

Renner *et al.* (7) provided additional information by developing an assay to measure sorbic acid in urine. They studied seven volunteers who ingested 1 mg/kg body mass of sorbic acid three times per day for 2 days and collected sequential 8-h urine specimens. They found increases in both sorbic acid and MA during the 2-day exposure period with mean 8-h MA peaks between 8,000 and 10,000 $ng \cdot g^{-1}$ creatinine kg^{-1} body mass.

Table 3 Predicted and reported MA values for specific air benzene levels

Study	Air benzene range (ppm)	1 ppm	0.5 ppm	0.1 ppm
Lauwerys *et al.* (28), 1994	0.03–2	1413 ng/mg	778 ng/mg	195 ng/mg
Ghittori *et al.* (29), 1996	0.01–0.3			194 ng/mg
	0.01–~1[a]	661 ng/mg	452 ng/mg	
Ong *et al.* (26), 1996	0.01–3.5	144.5 ng/mg	89.6 ng/mg	29.5 ng/mg
Hotz *et al.* (19), 1997	<0.01–7.33[b]		300 ng/mg	
Krivistö *et al.* (20), 1997	0.06–1	4530 ng/ml	2181 ng/ml	300 ng/ml
Javelaud *et al.* (30), 1998	<0.0015–2.9	291 ng/mg	208.5 ng/mg	96.1 ng/mg
Lagorio *et al.* (31), 1998	0.03–0.11			155 ng/ml (90 ng/mg)

[a] Three air benzene exposures were >1 ppm.
[b] 95% exposed <0.5 ppm.

If an average 70-kg individual is assumed, this corresponds to peaks of 560–700 ng/mg creatinine. They also found a correlation of 0.74 between sorbic acid and MA in 24-h urine collections from 69 volunteers.

Pezzagno *et al.* (4) summarized estimates for sorbic acid consumption by residents of different countries and areas that include 6–25 mg/day in Europe overall, 30 mg/day in the United States, and <100 mg/day in Italy. As noted above, ingestion of 60 mg of potassium sorbate (44.7 mg sorbic acid) in two volunteers resulted in MA peaks much lower than ours (6). Our results from actual foods are closer, on average, to those of Ducos *et al.* (21) after 200 mg of sorbic acid ingestion. This suggests that larger doses of sorbic acid were present in the foods ingested than were anticipated based on daily consumption estimates. This is certainly possible because suggested levels of sorbic acid-based preservatives in bakery products, for example, range from 0.03–0.3% (9). If the recommended 0.1% addition for yellow cake was present in the 3 oz. cupcake package, 84 mg of sorbic acid would be consumed. A 0.3% addition would result in ingestion of 252 mg of sorbic acid.

Other factors, such as interlaboratory differences and perhaps even the form of sorbic acid ingested, may be involved. For example, the two studies reporting 24-h MA excretion before and after administration of sorbic acid-based preservatives found average increases that differed by 2-fold. The study using a slightly higher dose of sorbic acid found an 11-fold increase in MA excretion (5), whereas a 23-fold increase was found in the study using a lower dose in the form of potassium sorbate (4). Timing of sample collection is another variable for studies in which MA from each urine void was measured. Samples collected frequently after ingestion have an increased potential to show greater concentrations. Although some concern has been raised regarding false-positive MA attributable to nonspecificity of the HPLC assay (22), we do not believe that this explains our results. Bartczak *et al.* (23) found a good correlation between MA measured by HPLC with diode array detection and gas chromatography-flame ionization detection. The variable MA response in ID 1 to different sorbic acid-based preserved foods is also unclear. Different forms of the preservative ingested may play a role.

There is a growing body of literature evaluating the association between MA and low-level ambient benzene exposure. Some authors have found excellent correlations. For example, Bergamaschi *et al.* (24) examined exposure in 24 nonsmoking bicyclists during 2-h rides on urban and rural routes. They measured benzene in air, blood, and urine. Urinary MA was also measured. A statistically significant correlation coefficient of 0.59 was found between air benzene (ranging from 1.2 to 26.1 ppb) and increase in MA pre- to post-ride. Others, however, have not found significant correlations. Examples include a study of 80 bus drivers, whose benzene exposure, based on urine benzene, was calculated to range from 3 to 313 ppb, in which urinary benzene and MA were not correlated (25), and a study showing lack of correlation between MA and air benzene at occupational exposure levels <0.25 ppm (26). In addition, several authors have reported finding occasional unexpectedly high MA levels (consistent with exposures to 1 ppm benzene) in nonoccupationally exposed control subjects (11, 25, 27).

Ingestion of preserved food varies by country. Therefore, it is likely that sorbic acid ingestion is a factor in the variable correlations between MA and other benzene exposure measures reported in the literature. It is also a likely explanation for the some of the elevated results seen in nonoccupationally exposed subjects. The levels of MA generated by ingestion of sorbic acid-containing foods are elevated to such an extent that this represents a source of confounding for occupational as well as environmental exposure. To use MA as a low-level benzene biomarker in countries with significant ingestion of sorbic acid-preserved foods, methods to avoid sorbic acid interference will need to be used. Potential solutions include simultaneously measuring urinary sorbic acid (7) and/or dietary restriction. An example of the latter approach is presently used in biomonitoring for arsenic. Prior to availability of arsenic speciation and even currently to reduce costs, seafood ingestion is restricted to avoid false positives attributable to organic arsenic ingestion. This approach for sorbic acid may be possible because, although several food types contain sorbic acid-based preservatives, the amount of these foods that is consumed in a sitting varies. Those that are consumed only in small quantities likely have less potential to result in significant sorbic acid consumption.

The issue of the most accurate way to adjust for differences in urine concentration is also raised by our data. The study included some males with relatively high urinary creatinines (>200 mg/dl). As shown in Table 2, large differences in MA results were obtained, depending on whether they were reported in ng/ml or ng/mg creatinine. In addition to concentration, numerous other factors, such as body mass, diet, and exercise, are known to influence urinary creatinine concentration. These sources of variation have been raised as limiting factors to the use of creatinine as an adjustment method for urine concentration differences (14). Creatinine adjustment results in lower adjusted values in males, on average, than in females or children who have lower creatinine excretion. Therefore, males could have higher actual exposures than would be apparent from adjusted values. An alternative method of adjustment for urine concentration may be useful when considering populations with potential for a wide range of creatinines.

In conclusion, our data indicate that specificity of MA as

a benzene biomarker may be compromised, even in occupational exposure settings, by ingestion of sorbic acid-based food preservatives. This is a particular concern in countries, such as the United States, where consumption of processed foods is common. To use MA as a benzene biomarker in countries with significant ingestion of sorbic acid-preserved foods, methods to address sorbic acid interference will need to be used. Additional research is required to determine whether dietary restriction and/or measurement of sorbic acid would be useful in this regard. In addition, our results suggest that the use of creatinine to adjust for spot urine concentration differences in age and gender diverse populations has limitations.

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Lack of Specificity of *trans,trans*-Muconic Acid as a Benzene Biomarker after Ingestion of Sorbic Acid-preserved Foods

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