

# Benzene Exposure Assessed by Metabolite Excretion in Estonian Oil Shale Mineworkers: Influence of Glutathione S-Transferase Polymorphisms

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## Abstract

Measurement of urinary excretion of the benzene metabolites *S*-phenylmercapturic acid (*S*-PMA) and *trans,trans*-muconic acid (*t,t*-MA) has been proposed for assessing benzene exposure in workplaces with relatively high benzene concentrations. Excretion of *S*-PMA and *t,t*-MA in underground workers at an oil shale mine were compared with the excretion in workers engaged in various production assignments above ground. In addition, possible modifying effects of genetic polymorphisms in *glutathione S-transferases* T1 (*GSTT1*), M1 (*GSTM1*), and P1 (*GSTP1*) on the excretion of *S*-PMA and *t,t*-MA were investigated. Fifty underground workers and 50 surface workers participated. Blood samples and three urine samples were collected from each worker: (a) a preshift sample collected the morning after a weekend, (b) a postshift sample 1 collected after the first shift, and (c) a postshift sample 2 collected after the last shift of the week. Personal benzene exposure was  $114 \pm 35 \mu\text{g}/\text{m}^3$  in surface workers ( $n = 15$ ) and  $190 \pm 50 \mu\text{g}/\text{m}^3$  in underground workers

( $n = 15$ ) in measurements made prior to the study. We found *t,t*-MA excretion to be significantly higher in underground workers after the end of shifts 1 and 2 compared with the corresponding surface workers. The same picture, although not significant, was seen for *S*-PMA excretion. Excretion of *S*-PMA and *t,t*-MA was found to increase significantly during the working week in underground workers but not in those employed on the surface. Both *t,t*-MA and *S*-PMA excretion were significantly higher in smokers compared with nonsmokers. Subjects carrying the *GSTT1* wild-type excreted higher concentrations of *S*-PMA than subjects carrying the null genotype, suggesting that it is a key enzyme in the glutathione conjugation that leads to *S*-PMA. The results support the use of benzene metabolites as biomarkers for assessment of exposure at modest levels and warrant for further investigations of health risks of occupational benzene exposure in shale oil mines. (Cancer Epidemiol Biomarkers Prev 2004;13(11):1729–35)

## Introduction

Benzene is defined as carcinogenic in humans, especially related to bone marrow toxicity and leukemia (1, 2). The risk of developing leukemia has been estimated to be ~6 cases per 1 million among people who experience lifelong exposure to benzene concentrations of  $1 \mu\text{g}/\text{m}^3$  in air (3). The general population is exposed to benzene in the outdoor environment through inhalation of polluted air mainly from gasoline-driven vehicles as well as from diesel exhaust (4). In the indoor environment, tobacco smoke is the main benzene

source, and benzene has been estimated to be responsible for one-tenth to one-half of smoking-induced total leukemia mortality (5). Occupational exposure to benzene is frequent such as in road tanker drivers (6) and Chinese glue and shoemaking factory workers (7). Biomarkers of internal benzene exposure, such as urinary excretion of the benzene metabolites *trans,trans*-muconic acid (*t,t*-MA) and *S*-phenylmercapturic acid (*S*-PMA) have been found to correlate well with external benzene exposure in several of these occupationally exposed groups (6, 7). Moreover, these metabolites represent the pathways leading to the formation of the putative toxic metabolites inducing bone marrow damage and other effects (8–11). This makes biological monitoring of benzene metabolites an attractive alternative in assessing benzene exposure in workplaces with relatively high benzene levels (e.g., >1 ppm or  $3.45 \text{ mg}/\text{m}^3$ ; refs. 7, 12, 13). However, with improved detection limits, this may also be possible at relatively modest exposure levels (e.g., <0.1 ppm; ref. 14).

The metabolism of benzene plays an important role in benzene toxicity (8, 9). Benzene is primarily metabolized

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in the liver via cytochrome P450 2E1 to benzene oxide, which can be further transformed to a variety of ring-hydroxylated and ring-opened metabolites (Fig. 1; ref. 8). Among these are *t,t*-MA and *S*-PMA formed during ring opening and ring hydroxylation pathways, respectively, after which they are both excreted in urine (Fig. 1). Several benzene metabolites are toxic. These include metabolites able to bind covalently to DNA and protein as well as quinone metabolites that can lead to generation of reactive oxygen species through redox cycling (10, 11).

A detoxification pathway of several intermediate benzene metabolites is conjugation by glutathione *S*-transferases (GST; Fig. 1; ref. 9). The GST classes M1 (*GSTM1*), P1 (*GSTP1*), and T1 (*GSTT1*) show genetic polymorphism, and an increased risk of several cancers have been found in individuals carrying the null genotypes (15, 16). With respect to benzene, the *GSTM1* wild-type was reported to confer increased signs of bone marrow toxicity in occupationally exposed subjects, suggesting that glutathione conjugation could increase toxicity (17). Another study found the excretion of the ring-opened metabolite *t,t*-MA to be increased in subjects with the *GSTT1* null genotype (18).

The aim of this study was to assess benzene exposure by measurement of internal dose biomarkers in mine-workers occupationally exposed to moderate levels of benzene and to investigate whether genetic polymorphisms in relevant metabolism enzymes influenced the excretion of these biomarkers. The urinary excretion of the benzene metabolites *S*-PMA and *t,t*-MA and the genotypes of *GSTM1*, *GSTT1*, and *GSTP1* were measured in 50 mineworkers and 50 surface workers.

## Materials and Methods

**Experimental Design.** This study was carried out at an oil shale mine "Estonia" in Kohtla-Jarve, northeastern Estonia, in June to July 2000. Mining was done by the pillar and chamber method, and by the time of the study, a workweek was composed of 3 to 4 production days. A total of 100 male subjects participated, of which 50 were underground workers who drove diesel-powered excavators and 50 were surface workers engaged in various production assignments aboveground not associated with the use of diesel-powered engines. The subjects were recruited by the mine safety organization. The local ethics committee approved the study protocol and the subjects gave written informed consent before entry into the study.

During the study, two questionnaires were used, both administered by interview. The first asked about age, job title, job history, smoking and dietary habits (consumption of grilled/broiled meat or fish), use of medication, and activities outside work that might cause exposure to benzene. The second focused on activities in the 24-hour period before each urine collection that might influence the excretion of *S*-PMA and *t,t*-MA (e.g., diet and use of open fire).

**Benzene Sampling and Analysis.** The measuring of benzene concentrations were done using passive samplers with carbon filters as sorbent for benzene (SKC Ltd., Dorset, United Kingdom), which have a sampling rate of 16 mL/min for benzene. Sampling commenced at

the start of a work shift and terminated at the end of a work shift, which summed up to an exposure time of ~8 hours. For personal exposure measurement, the passive samplers were placed in the breathing zone of 15 underground and 15 surface workers. The measurements were done prior to this study. At the same time, fixed passive samplers measured benzene concentrations during work shift: 15 underground samplers and 15 samplers in surface workrooms. The underground samplers were placed near positions where the underground bulldozers worked and at bulldozer cabins.

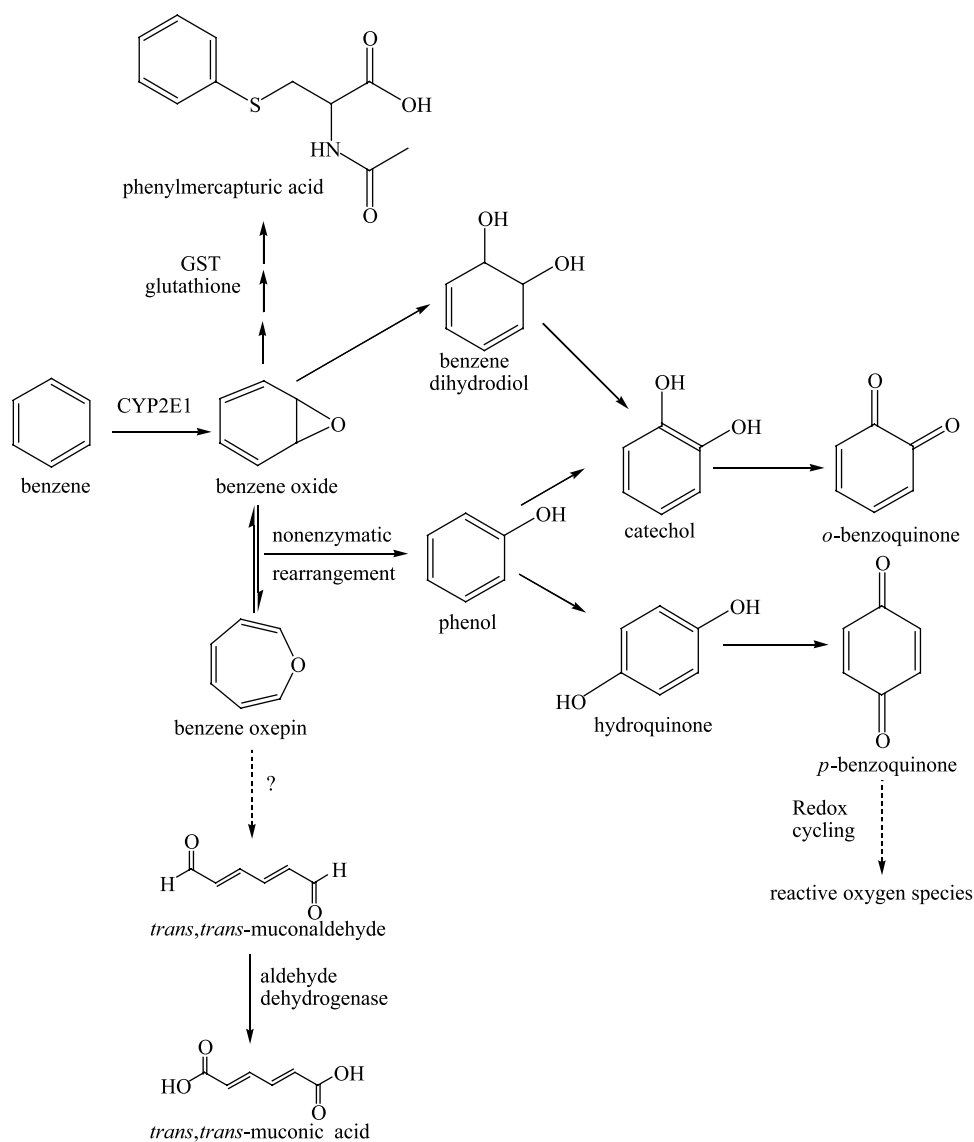
Benzene was extracted from the carbon filters by dimethylformamide, which has a recovery coefficient of 0.99. The benzene concentrations were subsequently determined by gas chromatograph (Chrom-5, Czech Republic) equipped with a flame ionization detector and a standard column DB-1, 30 m  $\times$  0.32 i.d. 1  $\mu$ m (Agilent Technologies, Palo Alto, CA).

**Urine and Blood Sampling.** All the workers delivered three spot urine samples in acid prewashed plastic containers. The first spot urine sample, the preshift sample, was delivered in the morning after a weekend without working in the mine. The second spot urine sample, postshift sample 1, was delivered in the afternoon of the first shift; the last postshift urine sample, postshift sample 2, was delivered after the last shift of the week, Wednesday and Thursday (19). The urine samples were immediately stored at  $-20^{\circ}\text{C}$  in Estonia and, after finishing the fieldwork, shipped to Denmark on dry ice where they were stored at  $-20^{\circ}\text{C}$  until analysis of *S*-PMA and *t,t*-MA.

Peripheral blood (8-10 mL) was collected in Vacutainer CPT tubes (Becton Dickinson, Franklin Lakes, NJ). The tubes were then gently tilted and centrifuged at  $1,650 \times g$  for 20 minutes. After removal of the plasma layer, the lymphocyte layer was collected and mixed with ice-cold RPMI 1640 with L-alanyl-L-glutamine (Life Technologies, Paisley, United Kingdom) to a total of 45 mL and centrifuged for 15 minutes at  $400 \times g$ . The supernatant was removed and the pellet was resuspended in ice-cold RPMI (25 mL). This lymphocyte suspension was centrifuged at  $400 \times g$  for 10 minutes at  $5^{\circ}\text{C}$ . Again, the supernatant was removed and the cells were resuspended in RPMI (15.5 mL) and stored in liquid nitrogen in Estonia and shipped to Denmark on dry ice where they were stored at  $-80^{\circ}\text{C}$  until analysis of GST genotype.

From the time of the collection, urine and blood samples were identified only by sample codes, and all laboratory analyses were carried out blind to the exposure status of the subjects.

**Trans-trans-Muconic Acid.** *t,t*-MA was measured in urine by gas chromatography-mass spectrometry based on a method published previously (20). In brief, urine (2 mL) was extracted in bond elute quaternary amine anion exchange cartridges (3 mL, Varian, Palo Alto, CA), washed with 1% acetic acid (3 mL), and eluted with 10% acetic acid (4 mL). The eluate was dried by vacuum. Then, 2-bromohexanoic acid (100 ng, Aldrich, Copenhagen, Denmark) was added to methanol as internal standard. The sample was reconstituted in boron fluoride (300  $\mu$ L) in excess methanol and derivatized at  $100^{\circ}\text{C}$  for 20 minutes. The sample was extracted with  $3 \times 1$  mL heptane and dried by vacuum until 50 to 100  $\mu$ L were left, and 1  $\mu$ L was injected on a Hewlett-Packard (Palo



**Figure 1.** Metabolic pathways of benzene.

Alto, CA) 6890 gas chromatography-mass spectrometry with a capillary column, 30 m, 0.25 mm diameter, cross-linked 5% PH ME soloxane (Hewlett-Packard). Sample injections were splitless. The initial column temperature was 80°C, which was maintained for 1 minute. Then, the temperature was raised at 12°C/min to 170°C (for 7.5 minutes) followed by 30°C/min to 280°C for 3.7 minutes. In the end of the run, the temperature was held for 1 minute at 280°C. The day-to-day interassay coefficient of variation was 4.7% and the intraassay coefficient of variation was 5.9%.

**S-Phenylmercapturic Acid.** S-PMA was measured in urine by gas chromatography-mass spectrometry based on a method published previously (12). In brief, urine (1 mL) was adjusted to pH 2, and S-benzylmercapturic acid (1 µg, Tokyo Kasei Organic Chemicals, Tokyo, Japan) was added as internal standard and extracted with ethyl

acetate (4 mL). After centrifugation at  $700 \times g$  for 10 minutes, the supernatant was dried by vacuum and then resuspended in 1.25 mol/L HCl (2 mL) in methanol. After derivatization for 30 minutes at 40°C, the samples were dried under a gentle stream of nitrogen at 45°C. The residue was resuspended in dichloromethane (150 µL) and 1 µL was injected on a Hewlett-Packard 6890 gas chromatography-mass spectrometry with a capillary column, 30 m, 0.25 mm diameter, cross-linked 5% PH ME soloxane (Hewlett-Packard). Sample injection was splitless. The initial column temperature was 35°C, which was maintained for 1 minute. Then, the temperature was raised at 12°C/min to 170°C followed by 30°C/min to 280°C for 3.7 minutes. In the end of the run, the temperature was held for 3 minutes at 280°C. Standard S-PMA was purchased from Tokyo Kasei Organic Chemicals. The day-to-day interassay coefficient of variation was 5.7% and the intraassay coefficient of variation was 7.7%.

**Table 1. Characteristics of the study participants**

	Surface workers (n = 50)	Underground workers (n = 50)
Age (y), median (range)	38 (20-54)	40 (24-54)
Minimum time in current occupation	5 mo	2 y
Weeks off work in the past 6 mo, median (range)	1 (1-10)	1 (1-12)
Cigarette smokers	31	34
Lubricating oil on hands most days at work	25	19
Grilled/broiled meat or fish at least weekly	8	5
Regular use of open fire	20	11

**Determination of *GSTT1*, *GSTM1*, and *GSTP1* Genotypes.** DNA was isolated from lymphocytes using standard phenol extraction procedures. The genotypes of *GSTT1*, *GSTM1*, and *GSTP1* were determined by PCR-based assays as described previously (21). For verification of the genotypes, an alternative assay for *GSTM1* and *GSTT1* was done on all samples by real-time PCR on a LightCycler (Roche, Mannheim, Germany) based on a method published previously (22). For *GSTM1* genotyping, the primers and incubation times were the same as described previously (22), whereas for *GSTT1* genotyping the following primers were used: forward primer: 5'-CATCTCCTTAGCTGACCTCGTAG-3' and reverse primer: 5'-GAAGTCCTTGGCCTCAGAA-3'. Denaturation was carried out at 95°C for 10 minutes. For both *GSTM1* and *GSTT1*, SYBR Green I was used as fluorescent probe and the final volume was 15 L. We found full agreement on the two methods of genotyping.

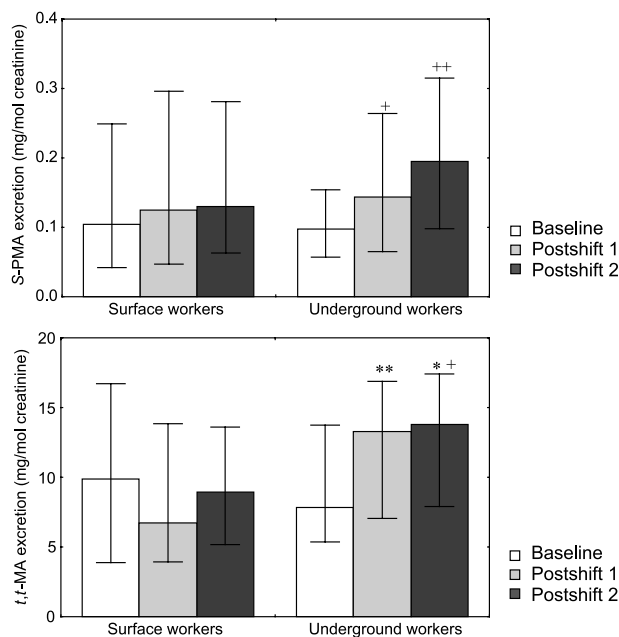
**Statistical Analysis.** Statistical analysis was carried out using Stata version 7.0 and SPSS version 9.0.1 software. Appropriate parametric tests of statistical significance (*t* test and ANOVA) were used, where a Shapiro-Wilk test indicated that the data conformed to a log-normal distribution. Otherwise, equivalent nonparametric tests were employed (Mann-Whitney test, Kruskal-Wallis test, and Cuzick's test for trend). Changes of within-subject *t,t*-MA or *S*-PMA excretion between baseline and postshift samples were analyzed using the

**Table 2. Personal benzene exposure of underground and surface workers and benzene concentrations at fixed surface and underground locations**

	Benzene concentrations ( $\mu\text{g}/\text{m}^3$ ), mean $\pm$ SD
Personal benzene exposure	
Surface workers*	114 $\pm$ 35
Underground workers*	190 $\pm$ 50 <sup>†</sup>
Fixed location benzene concentrations	
Surface*	130 $\pm$ 27
Underground*	290 $\pm$ 44 <sup>†</sup>
Ambient air, Kohtla-Jarve	29 $\pm$ 3

\*Based on results from 15 passive samplers.

<sup>†</sup>*P* < 0.05, significantly different from surface workers (*t* test).



**Figure 2.** Excretion rates of *S*-PMA and *t,t*-MA in surface and underground workers at baseline level and at the end of first shift (*Postshift 1*) and last shift (*Postshift 2*). Columns, median with interquartile range (Q25-Q75); *n* = 50 for all groups. \*, *P* = 0.05; \*\*, *P* ≤ 0.05, test for differences between surface and underground workers. +, *P* < 0.01; ++, *P* < 0.001, test for differences between baseline and postshift.

Wilcoxon signed rank test. The relationship between *S*-PMA and *t,t*-MA at each time point was analyzed using Pearson's correlation, where a logarithmic transformation satisfied a Shapiro-Wilk test of normality. A Hardy-Weinberg equilibrium test of *GSTP1* genotype distribution was done (23).

## Results

**Descriptive Data.** The 100 subjects participating in the study were between ages 20 and 54 years. They had all worked in their current occupation for at least 5 months. Sixty-five regularly smoked cigarettes, 44 got lubricating oil on their hands most days when at work, 13 ate grilled or broiled meat or fish at least once per week, and 31 regularly used an open fire that burned coal, coke, peat, or wood. Table 1 shows the distribution of these characteristics separately for surface and underground workers.

**Benzene Measurements.** Results on personal benzene exposures in surface and underground workers as well as concentrations of benzene at fixed locations at the surface and underground are presented in Table 2. The average personal benzene exposure was found to be 1.7-fold higher in underground workers compared with surface workers (*P* < 0.05) and the average benzene concentrations were 2.2-fold higher at fixed site locations underground compared with fixed location sites at the surface (*P* < 0.05) and 10 times higher than the levels in ambient air around the mine area.

**Table 3. The effect of smoking on urinary excretion of S-PMA (mg/mol creatinine) and *t,t*-MA (mg/mol creatinine)**

Cigarettes smoked per day	S-PMA			<i>t,t</i> -MA			<i>n</i>
	Baseline	Postshift 1	Postshift 2	Baseline	Postshift 1	Postshift 2	
0	0.05*	0.06*	0.07†	5.6*	5.1*	5.8*†	35
1-19	0.12	0.21	0.20	10.9	10.9	11.7	39
>20	0.20	0.22	0.29	13.8	15.4	14.0	26

NOTE: The data are given as medians.

\* $P < 0.001$ , regression test for trend.

† $P \leq 0.001$ , Cuzick's nonparametric test for trend.

**Urinary Metabolites of Benzene.** *t,t*-MA excretion was significantly higher in underground workers at postshift 1 compared with surface workers (adjusting for smoking status; Fig. 2). At postshift 2, *t,t*-MA was again higher in underground compared with surface workers (at borderline significance; adjusting for smoking). *t,t*-MA excretion at baseline and S-PMA excretion in all three time windows showed no significant differences according to workplace. The excretion of S-PMA and *t,t*-MA was found to increase significantly during the working week in underground workers but not in those employed on the surface. Both S-PMA and *t,t*-MA were higher in underground workers than in surface workers during the working week. In addition, both biomarkers were significantly higher in smokers compared with nonsmokers, especially in those who smoked most heavily (Table 3).

The relationships between excretion of S-PMA and *t,t*-MA at baseline level and at the end of postshifts 1 and 2 are illustrated in Fig. 3 (left, middle, and right). In all three time windows, a significant correlation was found.

**GST Genotypes.** The 100 participating subjects showed the following genotype distribution: for *GSTM1*, 62% were wild-type and 38% were null-type; for *GSTT1*, 86% were wild-type and 14% were null-type; and for *GSTP1*, 42% were a/a, 47% were a/b, and 11% were b/b. The distribution of genotypes in *GSTP1* was found to be in Hardy-Weinberg equilibrium. The *GSTT1* genotype was associated with postshift excretion of S-PMA; subjects with *GSTT1* wild-type excreted significantly higher concentrations of S-PMA during the first ( $P < 0.05$ ) and second ( $P < 0.001$ ) working shifts than subjects carrying the *GSTT1* null genotype (Fig. 4). There were no significant differences in *t,t*-MA excretion between the two *GSTT1* genotypes, and neither *GSTM1* nor *GSTP1* were associated with differences in excretion of S-PMA or *t,t*-MA.

## Discussion

Biological monitoring of benzene is an attractive way of assessing benzene exposure in workplaces and the present study shows that this applies to even modest exposure levels of 100 to 300  $\mu\text{g}/\text{m}^3$  or <0.1 ppm. Significantly higher excretion of *t,t*-MA was seen in underground workers, where the highest benzene exposures were measured, compared with surface workers. In addition, the excretion of S-PMA and *t,t*-MA increased significantly during the working week in underground workers and not in those employed on

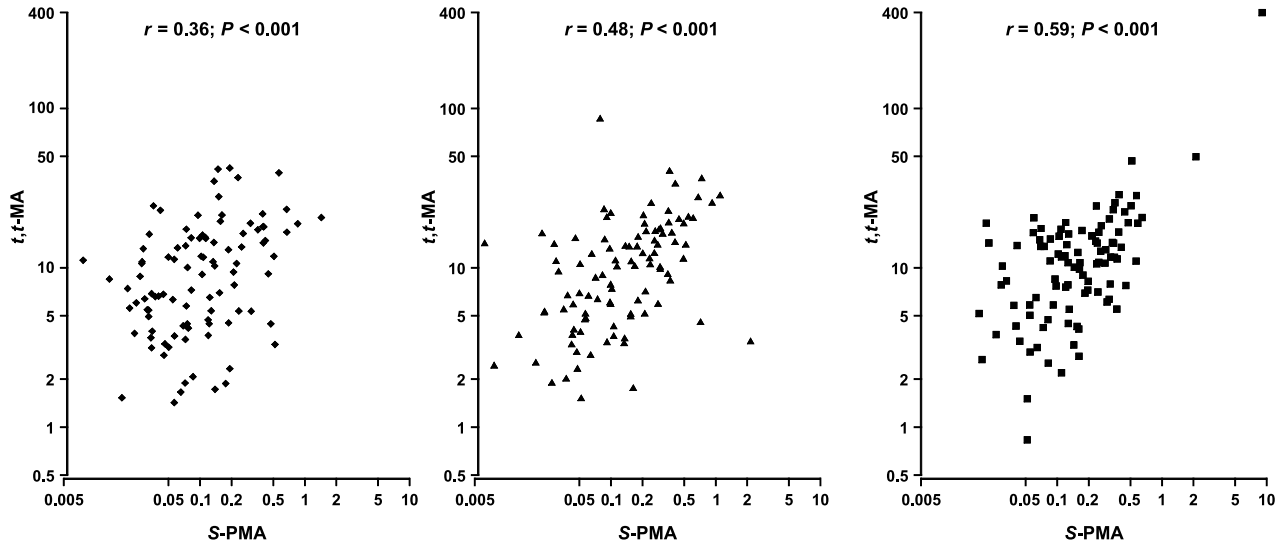
the surface. The excretion of S-PMA was dependent on the *GSTT1* genotype, as significantly lower levels were excreted following the first and second postshifts in subjects carrying the null genotype compared with subjects carrying the wild-type.

Benzene concentrations in the mine were  $\sim 2$  times higher than at various production sites at the surface in measurements prior to our study.<sup>5</sup> A possible explanation to this is that the shale stone contains benzene that could be released in the mine. Combined with a low ventilation capacity in the mine, this could lead to the increased benzene concentrations underground. In the mine, only diesel-powered trucks operated. Although gasoline-driven vehicles are the main contributors to airborne benzene in an outdoor urban environment, diesel exhaust is also known to contain benzene (4), which could help explain the increased benzene concentrations in the mine. However, whether evaporation from the shale stone, diesel exhaust, or other sources were responsible for the high benzene exposure in the underground, personal benzene concentrations in excavation workers remain to be determined.

The benzene exposures measured (114  $\mu\text{g}/\text{m}^3$  in surface workers and 190  $\mu\text{g}/\text{m}^3$  in underground workers) were found to be much higher than benzene concentrations in ambient air in the Kohtla-Jarve area (29  $\mu\text{g}/\text{m}^3$ ) or in urban background concentrations (annual mean, central Copenhagen, 1998: 2.9  $\mu\text{g}/\text{m}^3$ ; ref. 24). However, higher exposures have been reported in other occupations such as road tanker drivers (1.88 mg/m<sup>3</sup>; ref. 6) and Chinese factory workers (98.9 mg/m<sup>3</sup>; ref. 7). No other study known to the authors has reported benzene concentration in shale mine or other mines, but the results found in this study suggest that benzene concentrations in mines could be a relevant risk factor and warrant for further study.

Measuring of S-PMA and *t,t*-MA excretion has been proposed as an alternative way of assessing benzene exposure. We found excretion of *t,t*-MA to be significantly higher in underground workers after the end of shifts compared with the corresponding surface workers. The same picture, although not significant, was seen for S-PMA excretion. As the benzene exposure in the underground workers were  $\sim 2$  times higher than in the surface workers, these results support the use of *t,t*-MA and S-PMA excretion as suitable biomarkers for

<sup>5</sup> V. Muzyka, personal information.

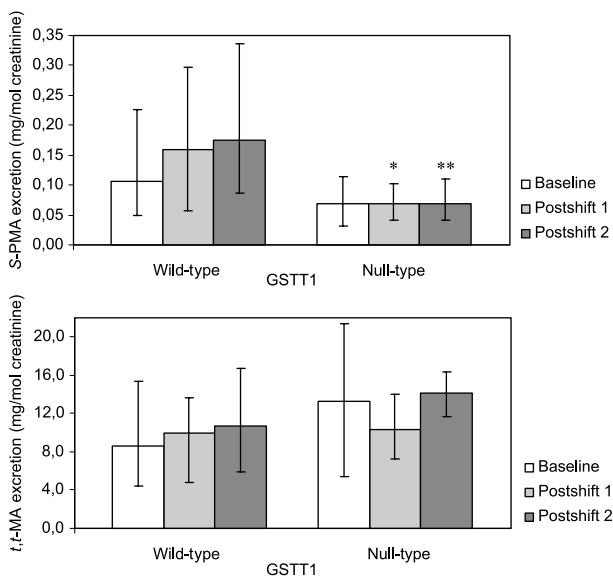


**Figure 3.** Relationships between the excretion of *S*-PMA and *t,t*-MA at baseline level (*left*;  $n = 100$ ), end of postshift 1 (*middle*;  $n = 100$ ), and end of postshift 2 (*right*;  $n = 99$ ). Pearson's correlation coefficient ( $r$ ) was calculated for each group.

benzene exposure at workplaces with relatively low benzene levels. Moreover, the excretion of *S*-PMA and *t,t*-MA increased significantly during the working week in underground workers and not in those employed on the surface. Finally, we found that excretion of *S*-PMA and *t,t*-MA showed a clear dose response in smokers,

corresponding to high concentrations of benzene in cigarette smoke (25).

Only a few studies have investigated the effect of genetic polymorphisms in metabolism enzymes such as GST on the excretion of *S*-PMA and *t,t*-MA. We found that following the first and second working shifts subjects carrying the *GSTT1* null genotype excreted significantly less *S*-PMA than subjects carrying the wild-type did, whereas there was no effect of the null genotype on *t,t*-MA excretion. As shown in Fig. 1, glutathione conjugation catalyzed by GST is a step in the metabolism pathway of benzene to *S*-PMA. However, the GST family responsible for this glutathione conjugation has not yet been identified. Our results suggest that *GSTT1* could be a key enzyme in this step. The fact that only *S*-PMA and not *t,t*-MA excretion is decreased in *GSTT1* null subjects indicates that the reduced *S*-PMA excretion cannot be explained by decreased exposure to benzene. Another study investigating the *GSTT1* genotype found that the null-type was associated with high *t,t*-MA excretion in bus drivers (18). However, it is difficult to imagine how GST genotypes could have a direct effect on *t,t*-MA excretion, as there is no obvious role for GST in the formation of *t,t*-MA. That study reported no effect of the *GSTT1* null-type on *S*-PMA excretion (18). As shown in Fig. 4, the *S*-PMA excretion is relatively constant in the *GSTT1* null-type subjects during the working week, whereas it is increasing during the working week for subjects carrying the *GSTT1* wild-type. The difference in *S*-PMA excretion between the two genotypes was only significant at postshifts 1 and 2 and not at baseline. This could indicate that differences in *S*-PMA excretion between the two *GSTT1* genotypes could only be distinguished at relatively high benzene exposures. The fact that benzene exposure in the bus driver study (18) was lower ( $82.2 \pm 25.6 \mu\text{g}/\text{m}^3$ ) than the exposures found in this study could explain why no



**Figure 4.** Excretion rates of *S*-PMA and *t,t*-MA according to *GSTT1* genotype at baseline level and at the end of first shift (*Postshift 1*) and last shift (*Postshift 2*). Columns, median with interquartile range (Q25-Q75);  $n = 90$  for all wild-type groups and  $n = 6$  (4 underground workers and 2 surface workers) for all null-type groups. \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ , test for differences between workers with *GSTT1* wild-type and null-type.

effect of *GSTT1* genotype was found in that study. Another aspect that differs between the two studies is the genotype distribution. In our study, only 14 (14%) subjects carried the null genotype, whereas in the bus driver study 15 (25%) subjects were *GSTT1* null. The *GSTT1* null genotype frequency in a Caucasian population is estimated to be 13% to 25% (26), suggesting a healthy worker effect (27) in the present study.

In conclusion, the results in this study support the use of excretion of benzene metabolites as biomarkers of internal dose of benzene in occupational settings and warrant further investigation of the health effects of occupational benzene exposure in shale oil mines. The special importance of *GSTT1* is seen by lower excretion of *S*-PMA in subjects carrying the null genotype, suggesting that it is a key enzyme in the glutathione conjugation that leads to *S*-PMA.

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