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# BIOMARKERS OF EXPOSURE IN MONDAY MORNING URINE SAMPLES AS A LONG TERM MEASURE OF EXPOSURE TO AROMATIC DIISOCYANATES.

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**Abstract**

*Purpose* Exposure to diisocyanates is a known occupational hazard. One method for monitoring occupational exposure is by analyzing biomarkers in hydrolysed urine and plasma. The half-life of the biomarkers in plasma is about three weeks, and the urinary elimination is divided in one fast (hours) and one slow phase (weeks). Polymorphism in glutathione S-transferase enzymes (GST) is earlier shown to modify the metabolism. The aim of the study was to assess if biomarkers of exposure in urine collected after two non-exposed days correlate to levels in plasma and if they can be used as a measure for long-term exposure to aromatic diisocyanates and further if polymorphisms in GST influenced the correlations. *Methods* Biomarkers of exposure was analysed in urine and blood samples collected from 24 workers, exposed to at least one of toluene-, methylenediphenyl- or naphthalene diisocyanate, on a Monday morning after at least two unexposed days. Moreover genotype was determined for 19 of the workers. *Results* The corresponding specific gravity adjusted biomarkers in urine and plasma levels for the different diisocyanates correlated well (r between 0.689 and 0.988). When taking all samples together the correlation coefficient was 0.926. Polymorphism in the *GSTM1* genotype seemed to modify the association. *Conclusion* Urine collected after two unexposed days can possibly be used as long term biomarker of exposure for aromatic diisocyanates.

Word count 217.

Key words: Occupational, isocyanate, biomarker, TDI, 4,4´-MDI, 1,5-NDI

## INTRODUCTION

Occupational exposure to diisocyanates is since long a well known occupational hazard (Swensson et al. 1955) but still common. In a recently published survey, exposure to isocyanates was the most frequent cause of reported occupational asthma in an industrialized part of UK (Bakerly et al. 2008). Accordingly, the occupational exposure limits to diisocyanates are low (SWEA 2011).

There are several different methods to monitor occupational exposure to diisocyanates. As the occupational exposure limits for diisocyanates are set as concentrations of the diisocyanates in air, environmental monitoring in air is still the most frequent way to monitor the exposure. Methods for biological monitoring, based on hydrolysis of urine or plasma and analysis of the corresponding amine, have been used since the mid eighties (Rosenberg and Savolainen 1986). High correlations between air levels of diisocyanates and levels of the corresponding diamines as biomarkers in hydrolysed urine have been shown both experimentally and in work environment for toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI) and isophorone diisocyanate (IPDI) (Maitre et al.1993; Tinnerberg et al. 1995; Maitre et al. 1996; Kääriä et al. 2001a; Liu et al. 2004; Sennbro et al. 2004). However, for 4,4´-methylenediphenyl diisocyanate (4,4´-MDI) and 1,5-naphtalene diisocyanate (1,5-NDI) low or no correlations have been seen between air levels and urinary biomarkers (Kääria et al. 2001b; Sennbro et al. 2006) indicating either another uptake or another metabolism.

In a recent publication (Broberg et al. 2010) on TDI it was shown that genetic polymorphisms, especially in glutathione S-transferase P1 (*GSTP1*), strongly modify the metabolism of this diisocyanate. A genetic effect modification was also seen for GSTM1 and indicated for GSTT1. This explains part of the inter-individual variations in biomarker levels observed (Sennbro et al. 2004) for TDI. Genetic variants that result in metabolic differences influence the levels of biomarkers, and the estimation of exposure in biomonitoring (Broberg et al. 2010).

When diisocyanates are inhaled they react with biomolecules (Sennbro et al. 2003). In the blood it has been shown that the diisocyanates are mainly bound to serum albumin (Lind et al. 1997a; Johannesson et al. 2004) and the half-life of the adducts in plasma has been reported to be 21 days which is similar to that of albumin (Lind et al. 1997b). The adducts will be excreted in urine as low-molecular weight conjugates (Lind et al. 1996) when the proteins are degraded. These are analyzed as diisocyanate derived amines in hydrolysed fluids (Sennbro et al. 2003) or can be analyzed as such (Sabbioni et al. 2012).

The urinary elimination for TDI is divided in one fast phase with half-life of about 2-8 hours (Brorson et al. 1991; Lind et al. 1997b) and one slow phase with elimination half-life of several weeks (Lind et al. 1996; Lind et al. 1997b). It was suggested that the slow phase in urine corresponds to the elimination of degraded protein adducts (Lind et al. 1997b). Lind et al also showed that the correlation between the levels of biomarkers of exposure for TDI in plasma and urine became higher after increased length to the latest exposure. The urinary elimination for 4,4´-MDI and 1,5-NDI are less studied, but in one study the urinary half-life for 4,4´-MDI was calculated to be between 59 and 82 hours. This calculation included both exposure and exposure free time (Dalene et al. 1997). In a recent study (Budnik et al. 2011) showed, after challenge exposure up to 120 minutes, that the elimination kinetics for different diisocyanates were very different. The peak time of urinary elimination was much faster for TDIs (about 4 h) than for 1,5-NDI (6 h) and 4,4´-MDI (14h). Moreover, an elevated urinary peak was seen for 1,5-NDI after more than 48 hours, although the numbers of observations were very small. Aliphatic isocyanates are not included in this study.

Protein adducts have been used as long-term exposure biomarkers for many compounds, mainly carcinogens (Törnqvist et al. 2002). Since the levels of protein adducts are related to the DNA adducts it has been suggested that the protein adducts better show the risk of the exposure to carcinogens than monitoring of metabolites only. In addition, studies of exposure-response relationships for airway diseases using protein adducts have been performed for aromatic diisocyanates (Littorin et al. 2000; Littorin et al. 2007) and organic acid anhydrides (Rosqvist et al. 2003) showing that the analysis of adducts are valuable tools in studies of such health effects. These protein adducts may have a large advantage since in many cases it is the generation of the protein adduct itself that starts the disease. For the organic acid anhydride this it is true (Nielsen et al. 1994) but remains to be shown for the diisocyanates.

If the slow elimination phase in urine indeed corresponds to the elimination of adducts, the analysis of the levels of amines in hydrolysed urine after an exposure-free period of time would be possible to use as a long-term biomarker of exposure instead of analysing the protein adducts in blood. In the present study, the main objective was to assess if urine samples collected after at least two non-exposed days are correlated with the protein adducts in blood and thus can be used to measure long-term exposure to aromatic diisocyanates. Furthermore, the aim was to study if polymorphism in glutathione S-transferase enzymes (*GSTM1*, *GSTP1* and *GSTT1*) influences the correlations.

## SUBJECTS AND METHODS

### Plants, workers and airborne exposure

The present study included 24 exposed workers from three plants in southern Sweden. The plants were two molding plants and one continuous foaming plant. Personal air samples were collected using a modified 2MP method with double filters impregnated with 1-(2-methoxyphenyl)piperazine (Henricks-Eckerman et al. 2002).

Plant A manufactured semi-flexible polyurethane (PUR) foam blocks by molding. The isocyanates used were mainly TDI, but 4,4´-MDI and IPDI were also used. The manufacturing process was divided in two steps. In the first step, the ingredients were mixed and then molded in an enclosed system. After hardening the PUR-material were moved to a heated water bath for expansion. There were six workers at each shift and one shift participated in the study. The measured personal airborne exposure was between 0.52 and 4.1 μg/m$^3$ for 2,4-TDI, 0.21 and 1.5 μg/m$^3$ for 2,6-TDI and between 0.02 and 0.39 μg/m$^3$ for 4,4´-MDI.

Plant B manufactured TDI-based flexible foam in continuous foam blocks in an enclosed ventilated tunnel system. There were six workers directly involved in the process and all six participated in the study. The foaming process took about 2 hours per day, during the rest of the day the workers were occupied with other tasks. During the foaming the workers wore personal protective equipment. The measured personal airborne exposure outside the personal protective equipment during foaming was between 0.40 and 29 μg/m$^3$ for 2,4-TDI and between 3.6 and 58 μg/m$^3$ for 2,6-TDI. The rest of the day the airborne exposure were below 0.2 μg/m$^3$ for both 2,4- and 2,6-TDI.

Plant C manufactured 1,5-NDI-, 4,4´-MDI-, and TDI- based rigid PUR products. The dosing, mixing and molding of the PUR were performed manually, partly in ventilated hoods. After hardening of the PUR-products overnight, the PUR material was refined by grinding and turning. A total of 16 workers were employed at plant C and 12 participated in the study. Different isocyanates were used in different parts at the plant. Some workers worked mainly with one or two of the isocyanates, and others were more mobile and were exposed to all three diisocyanates but at lower levels. There were two workers mainly working with TDI, two mainly with 4,4´-MDI and one with 1,5-NDI, the others were secondary exposed. The measured personal airborne exposure was between < 0.01 and 0.14 μg/m$^3$ for 2,4-TDI, 0.01

and 0.27 μg/m$^3$ for 2,6-TDI, between 0.04 and 9.7 μg/m$^3$ for 4,4´-MDI and between 0.03 and 30 μg/m$^3$ for 1,5-NDI.

The present study was approved by the Ethical Committee at Lund University, Sweden, and was performed with written informed consent of the workers.

**Collection of samples**

Urine and plasma samples were collected on a Monday morning before the start of the shift, when the workers had been unexposed for at least two whole days. The blood samples were collected by arm vein puncture, in Venoject® blood sampling tubes containing heparin. After arrival to the laboratory, the plasma was separated from the blood cells by centrifugation. The biological samples were then stored at -20°C until the day of analysis.

**Analysis of biomarkers**

The biomarker levels in the urinary and plasma samples were analyzed according to Sennbro et al. (2003). In principle, the biological samples were hydrolyzed for 24 h in 0.3 M NaOH in order to release the diisocyanate-related diamines 2,4-toluene diamine (TDA), 2,6-TDA, methylene dianiline (MDA) and naphthalene diamine (NDA). The diamines were extracted with toluene and after derivatization with pentafluoropropionic acid anhydride, the derivatives were quantified by gas chromatography and mass spectrometry. The limit of detection was 0.1 ng/ml for 2,4-TDA, 2,6-TDA and NDA in both urine and plasma and 0.05 ng/ml for MDA in both urine and plasma.

**Genotyping of glutathione S-transferases**

Out of the 24 workers participating in the study, genotype data was available for 19 of the workers. DNA was extracted from whole blood according to standard procedures. In all PCR assays, negative (containing water instead of DNA) and positive (DNA from individuals with known genotype) controls were included and 5% of the samples were rerun. The *GSTP1* polymorphism analysis results in an Ile to Val substitution at residue 105 in exon 5.The *GSTP1* genotyping method have been described in detail in Broberg et al., 2008. The *GSTM1* and *GSTT1* gene deletions were analyzed separately by TaqMan® Copy Number Assays on ABI 7900 instrument (ABI) following the instructions of the manufacture's protocol. The gene deletion polymorphisms result in three different genotypes: individuals carrying the gene on both chromosomes (2 copies), individuals with the gene on only one chromosome (one copy), and individuals with no gene present (null copy). The following probe were used for

detection of *GSTT1*: Hs00010004_cn, and for *GSTM1*: Hs02575461_cn (Applied biosystems). As an endogenous control RNAse P was run in the same analysis as either *GSTM1* or *GSTT1* (article number 4403326; Applied biosystems). The input of DNA in each assay was 8 ng and each individual sample was run two times. Included in each run were controls of the different genotypes for *GSTM1* and *GSTT1* analyzed previously with regular PCR and subsequent gel electrophoresis (Broberg et al., 2008). Samples with the genotype *GSTM1* null or the *GSTT1* null was used as a calibrator for each assay and the copy number was assessed by calculation of the ΔΔCt for each sample relative the calibrator sample.

**Statistics**

Only individual biomarker results above the calculated reference limits i.e. reference value for non-exposed workers (Sennbro et al. 2005) have been used in the calculations. The reference limits was calculated using the receiver operating characteristic (ROC) curve method (Altman 1991). Pair of samples (urine and plasma sample collected for the same worker) where both or one sample had values below the reference limits was not used. With this limitation, four of the workers did not add any data. Of these four, two workers had both the urinary and plasma levels of biomarkers below the reference limits; one had levels over the reference limit in urine but not in plasma and one had over the limit in plasma but not in urine. The reference limits used were for urinary 2,4-TDA 0.4 ng/ml, for 2,6-TDA 0.2 ng/ml, for NDA 0.2 ng/ml, for MDA 0.5 ng/ml and for plasma 2,4-TDA 0.1 ng/ml, for 2,6-TDA 0.2 ng/ml, for NDA 0.1 ng/ml and for MDA 0.4 ng/ml.

The correlation between the biomarkers in urine and plasma were analyzed by calculations of Pearson correlation coefficients (r). The calculations were performed for each diisocyanate and for all diisocyanates together i.e. the sum of all biomarkers in urine against the sum of all biomarkers in plasma from the same sample. The urinary biomarkers were calculated as uncorrected values and for both specific gravity and creatinine adjusted values. For specific gravity adjustment the average density of 1.016 was used (Boeniger et al. 1993).

The genotypes of *GSTM1* and *GSTT1* were analyzed according to the inferred phenotype: present (homozygotes and heterozygotes) vs. null, and the effect of *GSTP1* were assessed for IleIle+IleVal vs ValVal. Some individuals were exposed to more than one isocyanate, thus the measures for the levels of biomarkers in the graph for the genotypes are not statistically independent. The sum of biomarkers was calculated for individuals exposed to more than one diisocyanate and a graph was plotted to illustrate the possible genetic effect modification for total amount of biomarkers for the 19 individuals with available genetic

information.

For all statistical analyses, SPSS v.15.0 (SPSS Inc, Chicago, IL, USA) was used.

## RESULTS

The levels of biomarkers in hydrolyzed urine and plasma are displayed in table 1a and 1b. At company A all workers had levels of biomarkers of exposure for TDI in urine and plasma above the reference limits. None of the workers had biomarkers of 4,4´-MDI above the reference limits in the urine, but four had levels above the reference limits in plasma. At company B all workers had levels of biomarkers of exposure to TDI above the reference in plasma, but only three out of six workers had levels higher than the reference limits in urine for 2,4-TDI. At company C four of the twelve workers had too low values in either plasma or urine or both and therefore added no data to the analysis. One worker had levels of biomarkers above the reference limits for 2,4-TDI in both plasma and urine, and none for 2,6-TDI. For 4,4´-MDI, nine had levels of biomarkers above the reference limits in urine and nine in plasma but only seven were above for both biomarkers. For 1,5-NDI, nine had levels above the reference limits in plasma and six for urine but only five were above the reference limits for both biomarkers. In total there were 34 paired levels of biomarkers with levels above the reference limits.

The correlations between the biomarkers in plasma and in unadjusted, specific gravity adjusted and creatinine adjusted urine are displayed in table 2. For 2,4-TDI the correlations for the adjusted urinary levels were better than for the unadjusted, whereas for the other diisocyanates there were no large differences. In figure 1 a-c) the plasma and urine biomarkers are plotted against each other and were found to be well correlated, especially for the adjusted biomarkers in urine.

Two graphs displaying the possible effect modification are displayed in figure 2 and 3. The *GSTM1* genotype seemed to demonstrate an effect modification as the *GSTM1* null demonstrated higher concentrations in urine compared to *GSTM1* present at the same plasma levels, and this difference increased with higher diisocyanate exposure. For *GSTP1* ValVal demonstrated higher concentrations in urine compared to *GSTP1* IleIle+IleVal at the same plasma levels. No obvious pattern was seen for GSTT1.

## DISCUSSION

We have in this study shown that biomarkers of exposure to aromatic diisocyanates in plasma correlate well with adjusted urinary biomarkers after at least two days without exposure. This correlation is independent of type of aromatic diisocyanate. This finding further indicate that the aromatic diisocyanates probably act similar in the human body and what is excreted in urine reflects the aromatic diisocyanate content bound to the proteins that is degraded. Thus, to measure the corresponding amines in urine after two days without exposure can be seen as a measure of the aromatic diisocyanate exposure during a long period of time. Moreover, it seemed that in particular the *GSTM1* genotype modified the association between aromatic diisocyanates in urine and plasma. However, the modification was not so large at these exposure levels that it affects the use of the amines for long-term exposure measurements.

In this study we have only used levels of biomarkers above the reference limits in the calculations instead of above the detection limit. The rationale for this is that we wanted to be sure to use values that reflect the occupational exposure and not the background exposure and also that the levels of biomarkers in the urine mainly was from the slow elimination phase. We have earlier shown that non-exposed subjects have low levels of the analysed biomarkers of exposure for aromatic diisocyanates, especially for 4,4´-MDI (Sennbro et al. 2005). However, if using this method for exposure assessment, the assessor needs to be aware of the possibility of false positive as there can be a non-occupational exposure giving urinary biomarker levels above the reference limits, again especially for 4,4´-MDI.

There was a very high correlation between the adduct levels in plasma and the urinary levels regardless of aromatic diisocyanate. This is in accordance with the hypothesis of the study, i.e., the levels of metabolites in urine reflect the degradation of protein adducts. Thus, it is the rate of catabolism of the proteins that determine the levels in urine. However, the results

also suggest that the three different aromatic diisocyanates behave rather similar in the body, binding to the same proteins.

It seems to be some influence of the *GSTM1* and the *GSTP1* genotype on the relationship between the amines in urine and plasma. In our previous study we did find evidence of a similar effect of *GSTM1* on the relation between TDI in plasma and urine (Broberg et al. 2010). Moreover, we found a strong effect modification for *GSTP1*. In this study, there was a similar trend for the ValVal genotype displaying the fastest excretion. However, this effect is probably related to the fast phase of elimination of low molecular weight metabolites in the urine and not from degradation of proteins. It has been described that diisocyanates conjugates to glutathione, either directly or by the glutathione system, resulting in reactive conjugates (Day et al. 1997; Lantz et al. 2001). The half-lives of these conjugates seem to be rather short (Day et al. 1997). Thus, it is not probable that these exist in the body for as long as two days. On the other hand, Lind et al. (1997b) found rather varying half-lives of the amines in urine from workers. This might indicate that the diisocyanates are bound in different proportions to various proteins where the half-lives differ. The binding to different proteins might depend on whether or not diisocyanates are conjugated to glutathione. The extent of the conjugation may in its turn be influenced by polymorphisms in the glutathione S-transferase genes, which could explain our findings. This is an interesting hypothesis since the binding to different proteins might explain how these are related to health effects caused by diisocyanates (Broberg et al. 2008; Piirilä et al. 2001; Mapp et al. 2002). However, it should be stated that the influence of the glutathione S-transferase polymorphisms are not so large at the levels of exposure measured in this study, that it affects the use of the amines for long-term exposure measurements.

Several researchers argue that the main risk with isocyanates is peak exposures. If the peaks are the only relevant exposure then no biomarkers can be used in a proper way to

measure the risk. There are also reports on associations between plasma and urine levels of amines for aromatic diisocyanate exposure and symptoms of the eye and lower airways (Littorin et al. 2000; Littorin et al. 2007), supporting that there are also a risk with long-term exposures that can be measured with biomarkers in plasma and urine collected on Monday morning. We and others have previously shown that there is a good correlation between urinary biomarkers collected on one day and measured air levels of aromatic diisocyanates the same day. This means that collecting urine samples at other occasions mainly reflects the exposure that day and can not be used as a long-term marker.

One drawback with this method is that the levels in urine after two days without exposure will be low and that some exposed subjects will have levels of biomarkers in their urine below the reference limits. However, at company A were the measured airborne exposure to TDI in the order of a few micrograms and all studied workers had concentrations in the urine above the reference limit. The exposure at company A is about one tenth of the general Swedish occupational exposure limit (OEL) for diisocyanates at 2 ppb (SWEA 2011) and in relation to most other countries OEL´s at 5 ppb, the detection will be sufficient. The great benefit is that this sampling could be performed by the workers themselves and that it not is invasive.

The results from this study, if corroborated from future studies, can be used to design an alternative strategy to easy survey occupational exposure to aromatic diisocyanates. The strategy includes urinary sampling of workers before the shift starts after at least two exposure free days. The levels of biomarkers can be seen as a long term biomarker of exposure and with repeated measurements ie exposure trends can be studied and also workers with higher exposure can be found which can help to optimize preventive measures.

## CONCLUSION

Urine collected after two unexposed days, for example on a Monday morning, can possibly be used as long term biomarker of exposure to aromatic diisocyanates, irrespectively type of aromatic diisocyanate. Polymorphism in GSTM1 and GSTP1 seems to modify the metabolism but not to such an extent that the urine samples not can be used as long time markers.

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**Conflicts of interest** The authors declare that they have no conflict of interest.

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Fig. 1a-c. Associations between the urinary and plasma biomarker levels. □ = 1,5-NDI, ● = 2,4-TDI, ○ = 2,6-TDI, ■ = 4,4´-MDI a) Unadjusted levels in urine; b) Creatinine adjusted levels in urine; c) Specific gravity adjusted levels in urine.

Fig. 2. Scatter plot depicting the associations between biomarkers of isocyanate exposure, i.e., aromatic diamines in hydrolysed urine and in plasma. Influence of the *GSTM1* polymorphism is shown with regression lines for the different genotypes (*GSTM1* present is shown with O, *GSTM1* null homozygotes are shown with □).

Fig. 3. Scatter plot depicting the associations between biomarkers of isocyanate exposure, i.e., aromatic diamines in hydrolysed urine and in plasma. Influence of the *GSTP1* polymorphism is shown with regression lines for the different genotypes (*GSTP1* IleIle+IleVal is shown with O, *GSTP1* ValVal are shown with □).

Figure 1

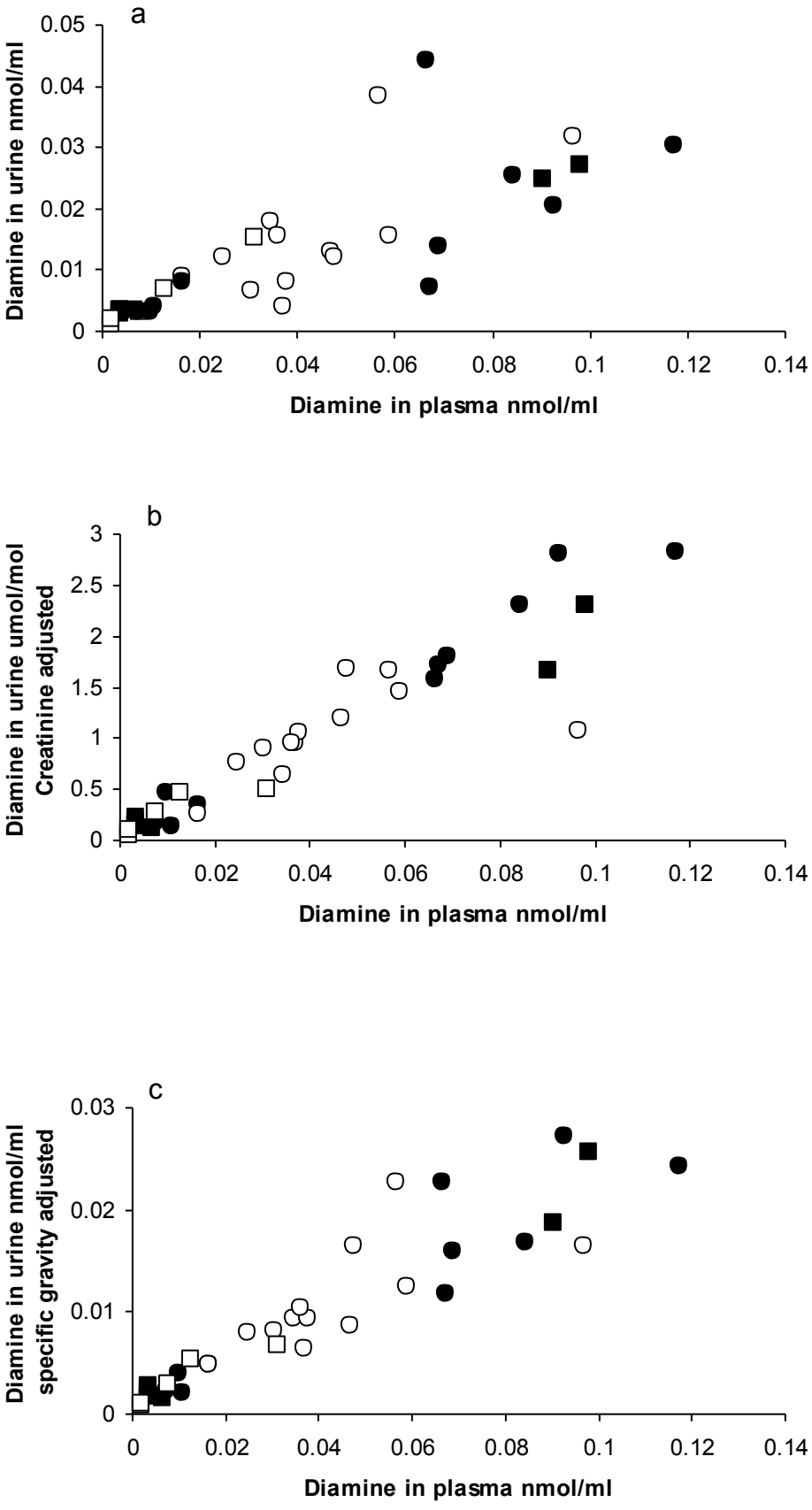
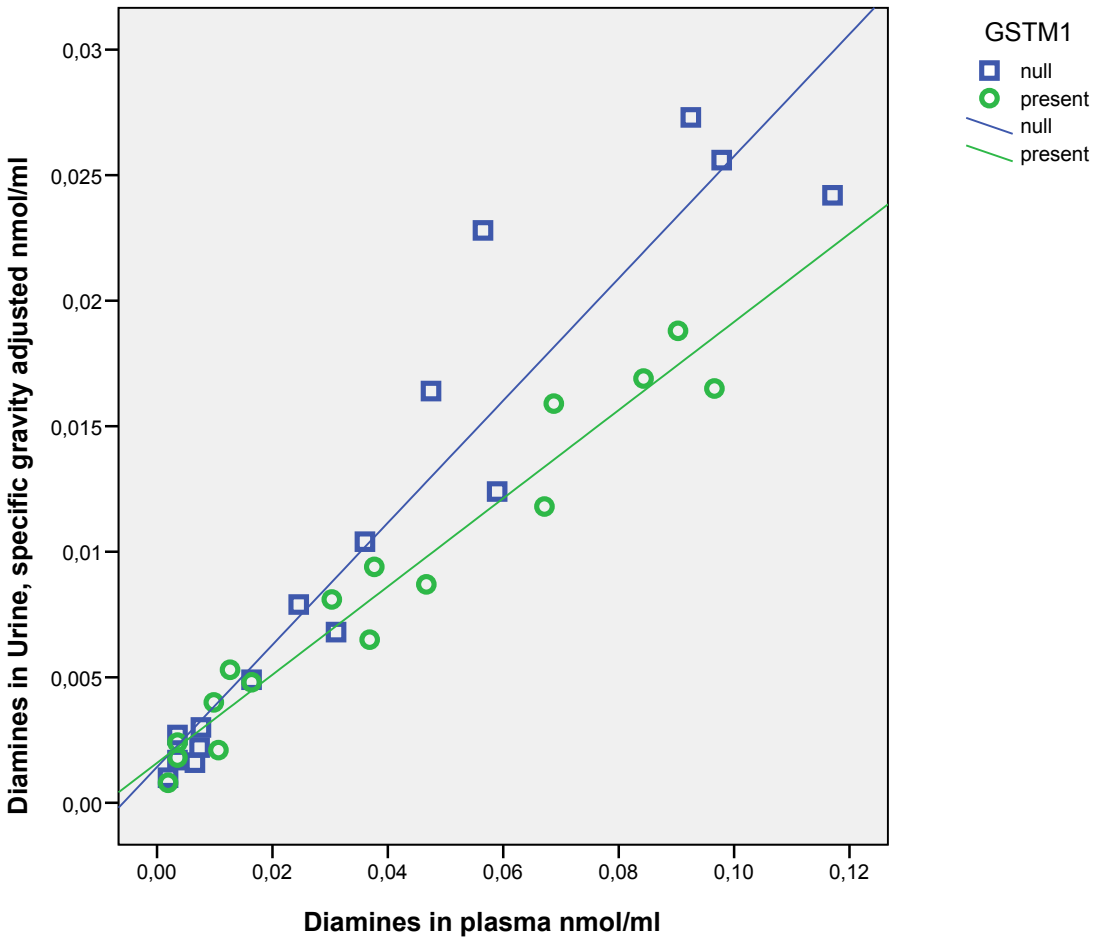

a
Diamine in urine nmol/ml
0.05
0.04
0.03
0.02
0.01
0
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
Diamine in plasma nmol/ml
b
Diamine in urine umol/mol
Creatinine adjusted
3
2.5
2
1.5
1
0.5
0
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
Diamine in plasma nmol/ml
c
Diamine in urine nmol/ml
specific gravity adjusted
0.03
0.02
0.01
0
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
Diamine in plasma nmol/ml

Figure 2.


GSTM1
null
present
null
present
Diamines in Urine, specific gravity adjusted nmol/ml
0,03
0,025
0,02
0,015
0,01
0,005
0,00
0,00
0,02
0,04
0,06
0,08
0,10
0,12
Diamines in plasma nmol/ml

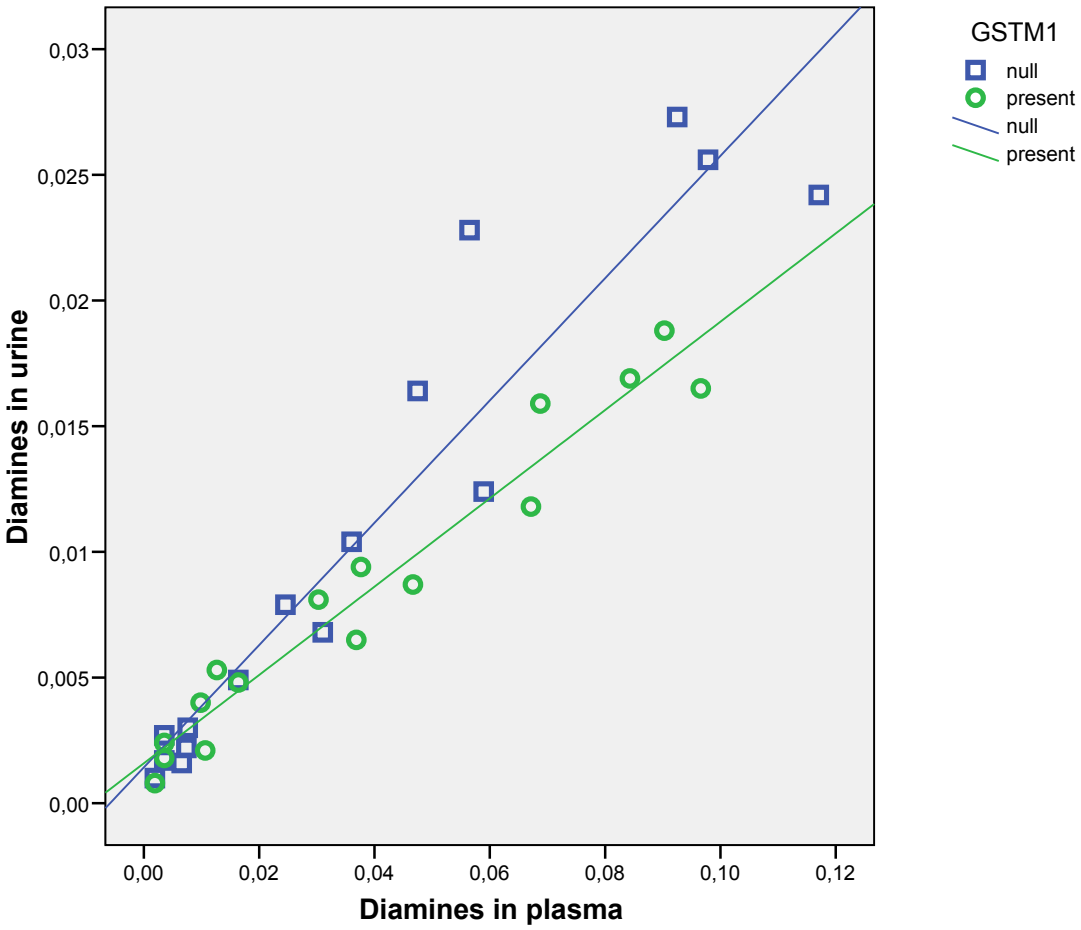

GSTM1
null
present
null
present
Diamines in urine
0,03
0,025
0,02
0,015
0,01
0,005
0,00
0,00
0,02
0,04
0,06
0,08
0,10
0,12
Diamines in plasma

Figure 3.

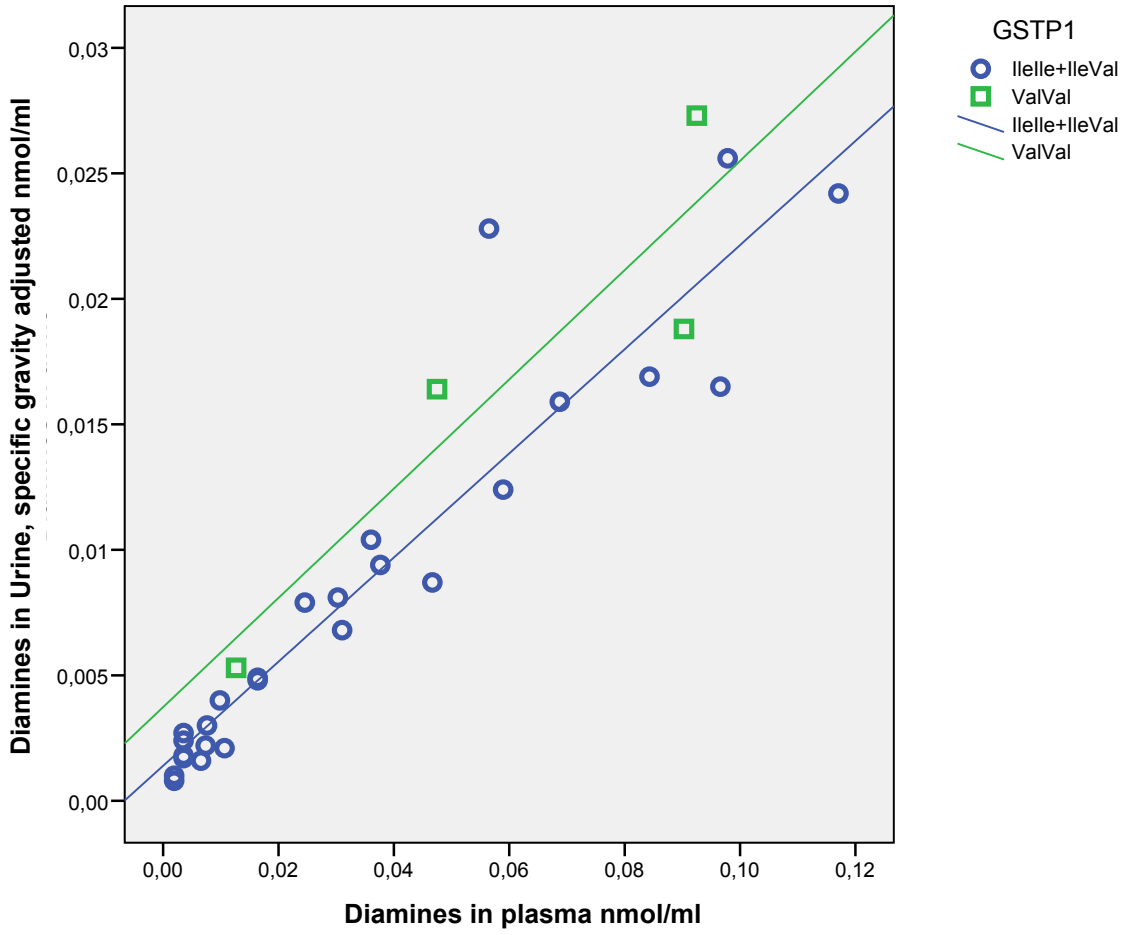

GSTP1
IleIle+IleVal
ValVal
IleIle+IleVal
ValVal
Diamines in Urine, specific gravity adjusted nmol/ml
0,03
0,025
0,02
0,015
0,01
0,005
0,00
0,00
0,02
0,04
0,06
0,08
0,10
0,12
Diamines in plasma nmol/ml

Table 1a. The unadjusted levels of biomarkers in the hydrolyzed urine above or equal the reference limits obtained in the different plants.

| Plant | Workers N | 2,4-TDA | | | 2,6-TDA | | | MDA | | | NDA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) |
| A | 6 | 6 | 2.8 | 0.9-5.4 | 6 | 1.6 | 0.5-2.2 | 0 | - | - | 0 | - | - |
| B | 6 | 3 | 0.5 | 0.5-1.0 | 6 | 1.7 | 0.8-4.7 | 0 | - | - | 0 | - | - |
| C | 12 | 1 | 0.4 | - | 3 | 0.2 | 0.2-0.2 | 9 | 0.7 | 0.5-8.4 | 6 | 0.4 | 0.2-2.4 |

Table 1b. The levels of biomarkers in the hydrolyzed plasma above the reference limits obtained in the different plants.

| Plant | Workers N | 2,4-TDA | | | 2,6-TDA | | | MDA | | | NDA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) | Number of workers above reference limit | Median (ng/ml) | Range (ng/ml) |
| A | 6 | 6 | 9.4 | 8.1-14 | 6 | 5.2 | 4.2-7.2 | 4 | 0.4 | 0.4-0.5 | 0 | - | - |
| B | 6 | 6 | 1.0 | 0.5-2.0 | 6 | 4.0 | 2.0-12 | 0 | - | - | 0 | - | - |
| C | 12 | 5 | 0.3 | 0.1-0.9 | 2 | 1.0 | 0.7-1.2 | 9 | 0.7 | 0.4-19.4 | 9 | 0.3 | 0.1-4.9 |

Table 2. Pearson correlations between the biomarkers in plasma and the biomarkers in unadjusted, specific gravity and creatinine adjusted urine.

| Diisocyanate | Number of observations | Unadjusted level in urine (nmol/ml) | Specific gravity adjusted level in urine (nmol/ml) | Creatinine adjusted level in urine (nmol/mol creatinine) |
|---|---|---|---|---|
| 2,4-TDI (nmol/ml) | 10 | 0.711* | 0.926** | 0.985** |
| 2,6-TDI (nmol/ml) | 12 | 0.690* | 0.689* | 0.557 |
| MDI (nmol/ml) | 7 | 1.000** | 0.988** | 0.986** |
| NDI (nmol/ml) | 5 | 0.995** | 0.929* | 0.856 |
| All (nmol/ml) | 34 | 0.798** | 0.926** | 0.933** |

** significant 0.01

* significant 0.05