

GENETIC POLYMORPHISMS AND BIOMONITORING OF STYRENE		
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Objectives

So far, biological monitoring methods and biological limit values applied in occupational and environmental medicine have been developed on the assumption that individuals do not differ significantly in their biotransformation capacities. It has become clear, however, that this is not the case but wide inter-individual differences exist in the xenobiotic metabolism. Modern techniques are now available to thoroughly address this issue. Integration of the data on individual metabolic capacity in biological monitoring studies is

anticipated to represent a significant refinement of the currently used methods. Consequently the present project aimed to clarify the potential role of the polymorphisms in *CYP2E1*, *EPHX1*, *GSTM1*, *GSTP1* and *GSTT1* genes in modifying individual responses to styrene, as measured by urine metabolites, adducts in blood macromolecules, and cytogenetic alterations in lymphocytes. It also aimed in developing alternative methods to assess *CYP2E1* phenotype, which would be more convenient for implementation in large epidemiological studies and in occupational medicine. The yet unclear relationship between *EPHX1* genotype and phenotype was also examined.

The following specific questions were studied:

- Effect of *CYP2E1*, *EPHX1*, *GSTM1*, *GSTP1*, and *GSTT1* genotypes on macromolecular adduct levels and on cytogenetic parameters in human whole-blood lymphocyte cultures *in vitro*.
- Effect of the genetic polymorphisms on internal dose by comparing styrene (and styrene oxide [SO]) concentrations in personal breathing-zone air samples with the levels of mandelic acid, phenyl glyoxylic and mercapturic acids in the urine of reinforced plastics workers representing different genotype constitutions.
- Effect of the genetic polymorphisms on the biologically effective dose of styrene by determining SO-adducts in haemoglobin and DNA of mononuclear blood cells in workers with known genotype constitutions.
- Effect of the genetic polymorphisms on the cytogenetic effects of styrene exposure by determining chromosome aberrations, micronuclei [utilising FISH (fluorescence in situ hybridisation)] in peripheral lymphocytes of styrene-exposed workers and control persons with known genotype constitutions.

Results

For the initial genotype screening, 100 male subjects, between 20 and 40 years of age, were recruited. They were asked to provide a blood sample for the genotyping analyses of XMEs. Based on this initial screening, 26 students were selected to obtain a balanced distribution between the variant alleles of *CYP2E1*, *GSTM1* and *GSTT1*. At this stage, their *GSTP1* and *EPHX1* genotypes were also determined.

Blood samples were collected before exposure for the measurement of *CYP2E1* mRNA content and *EPHX1* activity in PBLs, as well as for the measurement of background amount of ethanol and styrene in whole blood.

In the worker studies, four different worker cohorts were recruited from three different European countries, *i.e.*, Czech Republic, Italy, and Poland. Altogether 299 exposed workers and 167 unexposed controls were recruited during the study.

The results achieved so far indicate that genotyping and/or phenotyping of relevant XMEs does not significantly improve the interpretation of urinary levels of the main metabolites of styrene. However, *GSTM1* genotype appears as an important modifier of urinary PHEMAs excretion. Moreover, the mechanism by which GSTs catalyze GSH-conjugation of SO in humans seems to be regio- and stereo-selective. The [R,R]-M1 is the main mercapturate affected by the *GSTM1* genotype and it accounts, in the *GSTM1* positive subjects, for 56% of total PHEMAs excreted during the 24h following styrene exposure.

Although less significant than that of *GSTM1*, an involvement of *GSTT1* in the formation of PHEMAs is also possible. The influence of this genotype was specifically noted for the

[*R,R*]-M2 metabolite, further illustrating the regio- and stereo-selectivity of GST activity on SO.

A modifying role of *GSTM1* and *GSTT1* was also found regarding the induction of SCEs by styrene. Although glutathione conjugation is a minor route in human detoxification *in vivo*, individual sensitivity associated with the *GSTM1* and *GSTT1* null genotypes may be important locally in blood circulation and in blood-forming organs.

The observed significant relationship between urinary excretion of mandelic acid during 24 hours following styrene exposure and *CYP2E1* mRNA in PBLs demonstrates the importance of CYP2E1 in the metabolism of styrene in humans. On the other hand, based on the bio-transformation pathway of styrene in man, a combined effect of CYP2E1 phenotype and *GSTM1* genotype could be hypothesised to explain inter-individual variability in urinary PHEMA excretion. This could not be verified in the field study using the classic CZX metabolic ratio to assess CYP2E1 activity, but could be verified in the voluntary study where the CYP2E1 phenotype was assessed by *CYP2E1* mRNA levels in PBLs. This highlights the potential value of CYP2E1 phenotyping, through measurement of *CYP2E1* mRNA in PBLs, in *GSTM1* positive subjects. Therefore, while CZX may be considered as an 'acceptable' probe substrate for CYP2E1, a possibility exist that the CRM does not provide measure accurate enough to finely reflect the CYP2E1 catalytic activity.

Conclusions

Although genotyping and/or phenotyping of relevant XMEs does not seem to significantly improve the interpretation of urinary levels of the main metabolites of styrene, these analyses might greatly improve the interpretation of urinary concentrations of minor - but more specific - metabolites. In this respect PHEMA determination can now be considered as a useful tool for biological monitoring of styrene exposure in occupational or environmental settings. The first practical recommendation of this study is therefore to propose a systematic *GSTM1* genotyping to allow proper PHEMA data interpretation. Secondly, in *GSTM1* positive subjects excreting 'unexpected' amounts of PHEMA, the measurement of *CYP2E1* mRNA in peripheral blood lymphocytes could be recommended to discriminate between individuals who are really exposed to different levels and those who are more likely to be subject to host-associated factors influencing CYP2E1 expression, and hence, mercapturate production. This approach could be extended to other specific mercapturic acids, benzene, toluene, ethylene oxide, etc.), giving a new prospect for their use in the field of biological monitoring of exposure to chemical agents.

Future actions

In this study the reinforced plastics industry was used as a paradigm to examine the impact of polymorphisms on biomonitoring methods, but the results will offer the opportunity for more general applications in other areas of industrial and environmental medicine. If the results provide a way to identify individuals sensitive to the exposure, possible ill-effects related to the exposure might be prevented by improving working conditions. The economic and social impacts from improved occupational health are evident. Besides from the occupational health point of view, the sensitivity issue might have some bearing also in the general environment, which use similar biomonitoring approaches.