

Environmental monitoring and biomarkers of exposure to styrene in chemical industry

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ABSTRACT: Biomarkers of exposure to chemicals, such as styrene, allows to estimate the exposure to a particular compound by measuring the compound or it(s) metabolite(s) in body fluids. As an example, the determination of mandelic acid and phenylglyoxylic acid in urine. Indicators of genotoxicity are categorized as: (a) DNA and protein adducts; (b) DNA strand breaks. Styrene metabolism is initiated by Cytochrome P450 enzymes mediated by the oxidation of styrene to a reactive metabolite, the styrene-7,8-oxide. This study aims to review the environmental monitoring, complemented with biomarkers of exposure in order to understand its correlation and styrene genotoxicity. Systematic searches were performed to identify studies of occupational exposure to styrene and health effects in workers of chemical industries. To determine styrene air concentration, several studies used a personal air sampling method. A significant correlation has been found between styrene air concentration and mandelic and phenylglyoxylic acid concentration in urine. A significant relationship was found between individual levels of mandelic acid and phenylglyoxylic acid and styrene-7,8-oxide adducts to N-terminal valine in hemoglobin among exposed individuals. A strong correlation was also found between the frequency of DNA single-strand breaks, in mononuclear leukocytes, and styrene airborne level. The relationship between DNA damage, persistence and repair is complex, which complicates the relevance evaluation of potential genotoxic exposures difficult. There is conflicting evidence on the relationship between genotoxic response and exposure level. In future individual susceptibility studies, the individual genotypes associated with the metabolic route and DNA damage of styrene (metabolizing enzymes and DNA repair enzymes) should be analyzed. From the analysis of several studies it was found that indeed there is a strong correlation between the exposure levels and exposure biomarkers; however, no evidence was found regarding the styrene genotoxicity.

Keywords: Occupational exposure, Styrene; Chemical industry, Styrene; Environmental and biological monitoring, Styrene; Exposure biomarkers, Styrene

Monitorização ambiental e biomarcadores de exposição ao estireno na indústria química

RESUMO: Os biomarcadores de exposição a químicos, como o estireno, indicam que a exposição a um composto específico ocorre através da medição do composto inalterado ou do(s) seu(s) metabolito(s) nos fluidos corporais, como o ácido mandélico e o ácido fenilgloxílico presentes na urina. Os indicadores de genotoxicidade enquadram-se nas seguintes categorias: (a) adutos de proteínas e ADN; (b) quebras de ADN. O metabolismo do estireno é iniciado pelas enzimas do sistema citocromo P450, mediante a oxidação do estireno para um metabolito reativo, o 7,8-óxido de estireno. O presente estudo tem como objetivo a revisão de literatura relacionada com a monitorização ambiental do estireno e a existência de biomarcadores de exposição de forma a

compreender a sua correlação com a genotoxicidade do estireno. Realizaram-se pesquisas sistemáticas para identificar estudos distintos de exposição ocupacional ao estireno e os seus efeitos na saúde de trabalhadores de indústrias químicas. Em resultado dessa pesquisa verificou-se que vários estudos referem a utilização de amostragem de ar pessoal para a determinação da concentração de estireno. Uma forte e significativa correlação foi encontrada entre a concentração de estireno no ar e a concentração de ácido mandélico e o fenilglicoxílico na urina. Uma correlação significativa entre os níveis individuais de ácido mandélico e fenilglicoxílico e a presença de adutos de 7,8-óxido de estireno no N-terminal da valina da hemoglobina foi igualmente encontrada nos indivíduos expostos a concentrações mais elevadas de estireno. Estes adutos de 7,8-óxido de estireno e hemoglobina persistem durante a vida útil dos eritrócitos. Estudos revelam também uma forte relação entre a frequência de quebras da cadeia simples do ADN nos leucócitos mononucleares e o nível de estireno presente no ar. A relação entre as lesões, a sua persistência e a reparação do ADN é complexa, o que dificulta a avaliação da relevância de potenciais exposições a substâncias genotóxicas. Existem evidências conflitantes da relação entre a resposta genotóxica e o nível de exposição. Como tal, os estudos sobre a suscetibilidade individual devem analisar e associar os genótipos individuais com a via metabólica do estireno e a lesão no ADN (metabolizando enzimas e enzimas de reparação do ADN). Concluiu-se que existe uma forte correlação entre os níveis de exposição e os biomarcadores de exposição. No entanto, não foram encontradas provas quanto à genotoxicidade do estireno.

Palavras-chave: Exposição ocupacional, Estireno; Indústria química, Estireno; Monitorização ambiental e biológica, Estireno; Biomarcadores de exposição, Estireno

Introduction

Styrene and styrene plastics are indispensable to our modern world¹. It is a colorless or slightly yellow liquid, that evaporates easily with a vapor pressure of 867 Pa at 25 °C²⁻³ and it has a sweet smell⁴.

It is widely used in the production of polystyrene plastics, protective coatings, styrene polyesters, copolymer resins and as a chemical intermediate².

Some products that contain styrene are fiberglass, plastic pipes, automobile parts, shoes, drinking cups and other food containers, and carpet backing⁴.

Workers are exposed to styrene in several industries. Occupational exposures occur during racking and handling of liquid styrene¹, and so it is expected that production and maintenance workers are the most exposed at short-term⁵.

Immediate exposure may cause symptoms such as vision impairment, fatigue and drowsiness, increase of reaction time, reduced focus and balance problems⁴. Styrene is poorly soluble in water, but highly soluble in fat. It can bind to rich lipid tissues such as brain, myelin, and adipose tissue⁶⁻⁸.

Concerning the production of plastic and resins, 8-hour average samples in breathing zones often exceed styrene concentrations of 100 ppm⁹. The short-term effects start appearing at 20 ppm in 2-3 hours of exposure¹⁰. Some studies suggest that exposure to styrene concentration as low as 10 ppm may induce health effects².

Following the Portuguese Standard 1796, of 2014 September, the occupational exposure limits (OELs) for Portugal is established as 20 ppm during 8-hour exposure and 40 ppm for 15 min exposure¹¹.

The biomarkers indicate that exposure to a compound has occurred by measuring the compound or its metabolite(s) in

body fluids¹². Biomarkers of exposure can be separated into markers of effective dose, markers of effect and markers of internal dose¹².

Several studies described relations between the concentration in air and styrene metabolites levels in urine such as mandelic acid (MA) and phenylglyoxylic acid (PGA)¹³⁻¹⁶.

Styrene metabolism is initiated by cytochrome P450, this enzyme mediates the oxidation of styrene to a reactive metabolite styrene-7,8-oxide (SO)¹⁷. This intermediate is capable of binding covalently with macromolecules and is directly responsible for the genotoxic effects of styrene¹⁷. The genotoxicity of styrene depends on the ability of its epoxide, to interact with macromolecules including proteins and nucleic acids¹⁸.

The genotoxicity of styrene has been extensively studied¹⁸⁻²⁰. Indicators of potential genotoxicity fall into the following categories: (a) evidence that styrene exposure can increase the number of DNA and protein adducts; (b) evidence that styrene exposure can damage DNA by causing DNA strand breaks¹².

The carcinogenic potential of styrene stems largely from the ability of its metabolite, styrene-7,8-oxide, to bind covalently to DNA and from its activity in a variety of genotoxicity test systems²¹.

This study aims to review the environmental monitoring, complemented with biological exposure biomarkers to understand its correlation and styrene genotoxicity.

Methods

Systematic searches were performed (PubMed, selecting title, abstract, keywords) to identify studies of occupational

exposure to styrene and health effects, in chemical industries, using the following search terms:

- a) Occupational exposure to styrene – health effects;
- b) Environmental and biological monitoring;
- c) Biomarkers of exposure to styrene.

Studies were categorized as those that estimate: (1) environmental monitoring of styrene; (2) health impacts of occupational exposure to styrene; (3) assessing the occupational exposure with associations between biomarkers and environmental monitoring.

Twelve articles were selected by matching to our criteria and were excluded ten of the twenty-two analyzed; the studies that met our search criteria but did not provide information on occupational exposure to styrene, environmental monitoring and biomarkers were excluded.

Results

Environmental monitoring

Air samples are collected in activated charcoal tubes, then are analyzed using gas chromatography²²⁻²⁵ after desorption with 2ml carbon disulfide according to the NIOSH method²⁶⁻²⁸. However, several other studies claim that the concentration of styrene in the air can be determined by spectrophotometry²⁹⁻³² and polarography³³. Charcoal tubes are connected to battery-powered personal air sampling pumps operating at a flow rate of 100-200 ml/min^{27-28,40}.

To determine the atmospheric styrene, several studies used a personal air sampling, performed in the breathing zone (near the upper respiratory tract) for significant working periods^{27-28,40}.

Mandelic and phenylglyoxylic acids in urine

In humans, the main route of metabolism of styrene is through cytochrome P450, originating styrene-7,8-oxide³⁴⁻³⁶. The epoxide is further hydrolyzed to styrene glycol by epoxide hydrolase, which is then oxidized to MA and PGA by alcohol and aldehyde dehydrogenases¹⁹.

Following inhalation, the half-life for styrene is 41 min in blood³⁷ and 32-46 h in fat tissue³⁸. The next morning measurements reflect better past exposures due to the high fat solubility of styrene^{15,39}.

Several studies recommend that urine samples should be collected twice, in the next morning and after work shift^{12,40}. The urine samples should be analyzed on the day they were collected if possible, kept at 4 °C or more preferably at -20 °C⁴⁰⁻⁴¹.

Obtained by linear regression ($r \approx 0.9$), a strong and significant correlation has been found between the styrene air concentration and concentration of MA + PGA in urine^{2,28,40,42}. Only one study claims that all urine samples from control subjects were below the limit of detection of the analytical methodology used for MA and PGA determination⁴⁰.

A slightly significant relation was found between colour vision loss exams and the concentration of urinary MA and PGA². This colour vision loss is considered acquired dyschromatopsia, the effect on visual function related to styrene exposure in the workplace⁴³⁻⁴⁶. The damage to visual function could reflect neural alterations in the peripheral system or a result of damage to ocular structures⁴⁷⁻⁴⁸.

DNA adducts

Substances that bind to DNA can cause DNA replication errors during cell division (mitosis), which may then be propagated to additional lines of cells. The tendency of a substance to bind to DNA, to form 'DNA adducts', may be a useful indicator of potential carcinogenic activity for several reasons. The result may be the development of cancer, depending on the nature of those errors⁴⁹.

Adduct persistence indicates that the carcinogenic potential activity varies according to adduct binding site, because cells have mechanisms for repairing adducts. Predominantly adducts bind to guanine at positions N7, N2, and O6, when DNA is directly exposed to SO⁴².

In relation to controls (mean 13 pmol/g globin), increased levels of SO adducts to N-terminal valine in hemoglobin (SO-Hb) were observed in reinforced plastics workers (mean 28 pmol/g globin)⁵⁰.

A significant correlation was found between the individual levels of MA+ PGA and the SO-Hb among exposed individuals^{12,27}. A significant difference was also found in SO-Hb levels between workers with high exposure (average estimated was 24 ppm) and controls. However at lower levels of exposure (7 ppm), SO-adducts in N-terminal valine of hemoglobin were not detected⁵¹.

Between SO-adducts in N-terminal valine and concentration of styrene in the air, age and years of exposure no correlation was obtained. These factors do not seem to influence the SO-Hb adducts levels, because SO-Hb adducts persist during the lifespan of erythrocytes, and their level is closely related to the concentration of SO in circulation^{12,27}.

DNA breaks

The results obtained in styrene exposed workers studies suggest that styrene exposure leads to an increase of levels of DNA damage^{42,52-56}, with an increase in DNA strand breaks in leukocyte DNA related with the exposure of workers to styrene⁵⁷⁻⁵⁸.

A strong correlation has been described between the frequency of DNA single-strand breaks in mononuclear leukocytes and both the level of styrene exposure in the air and years of exposure to styrene¹².

The ability of SO and styrene to induce DNA strand breaks in mammalian cells has been well documented both in vitro^{52,59} and in vivo^{55,57}. However, because such breaks are quickly repaired, their significance as an indicator of toxicity is unclear⁵².

Table 1. Samplings characterization of the analyzed studies

		Sample	Indicator	References
Environmental Monitoring		Personal air sampling	Styrene air concentration	27, 28, 40
Biomarkers of exposure	Internal dose	Urine samples	Mandelic Acid (MA) phenylglyoxylic Acid (PGA)	2, 28, 40, 42
	Effective dose	Blood samples	DNA adducts (SO-Hb)	12, 27, 51
	Effect	Blood samples	DNA breaks	12, 52, 55, 57, 59

Discussion

Concerning to the environmental monitoring, the personal dosimeters obviously provide a more relevant exposure parameter on an individual basis than static monitors. Both of techniques lack in detecting exposure peaks, which may be the critical factor in the induction of biological effects when the average workplace styrene levels are kept within exposure limits⁶⁰. That is, if styrene were genotoxic *in vivo*, then it would be expected that studies with higher levels of exposure would be more likely to be positive and that different levels of response would occur in relation to different exposures inside a study population⁶⁰.

The measurements of MA and PGA are routinely used as biological control²⁷ of styrene exposure worldwide¹². These metabolites are rapidly changed and thus can only be used to indicate recent exposure and give the most precise information of individual's exposure and consider the personal protection equipment⁶⁰.

Overall, for humans, 2.6% of absorbed styrene is excreted in an unchanged state in urine, 85% as urinary MA and 10% as urinary PGA and 5% in the exhaled air⁶¹.

The concentration of MA in urine that corresponds to an 8h exposure to 50 ppm (213mg/m³) styrene would be expected to be 800-900mg/g creatinine at the end of a shift and 300-400mg/g creatinine the following morning^{14,16,62}. The PGA concentration in urine that corresponds to an inhalation of 50 ppm styrene for 8h would be approximately 200-300mg/g creatinine at the end of a shift and about 100mg/g creatinine the following morning^{16,61}.

A significant correlation was found between styrene concentrations in inhaled air and its urinary metabolites^{27,42,63}. Furthermore, results showed a more significant correlation with styrene concentrations in inhaled air if the urine samples were collected in the morning following the exposure to measure MA and PGA, than the determination of MA alone, which is usually undertaken after the work-shift^{15,64}.

It was observed that SO-Hb adducts were significantly higher in exposed subjects as compared to controls and correlated with internal dose parameter (MA and PGA) accor-

ding with some previous studies^{27,50,66}. A very significant correlation between styrene concentrations in the workplace, styrene metabolites in urine and SO-Hb adduct measurement becomes a means of assessing exposure to styrene at occupational and environmental level²⁷.

The DNA break provides information about temporary strand break. In normal circumstances, it gets repaired in a few hours before being fixed as a mutation¹². The relationship between DNA damage, persistence and repair is complex and complicates the evaluation of the relevance of potential genotoxic exposures. It is likely that there is a level of DNA damage which does not have any biological significance due to repair but there is no understanding of what this level is for individual genotoxic⁶⁰.

There is conflicting evidence for a relationship between genotoxic response and exposure level⁶⁰. Furthermore, the genotoxic potential of styrene in humans is predicted to be lower than in rats and mice due to a lower rate of SO formation from styrene and a more efficient conversion of SO into water-soluble metabolites, resulting in lower blood levels of SO⁶⁷.

Because of the inconsistent and predominantly weak effects which have been reported for the genotoxicity of styrene in humans, several studies investigate the influence of genetic polymorphisms to find whether styrene is an *in vivo* mutagen through the possible identification of susceptible individuals with higher levels of damage due to their differences in activation and inactivation of styrene⁶⁰.

Genetic diversity is clearly important in influencing individual susceptibility to carcinogens¹². Surely, gene-gene interactions and gene-environment interactions are fundamental to the interpretation and use of susceptibility biomarkers, so studies of individual susceptibility should analyze the individual genotypes associated with the metabolic route and DNA damage of styrene (metabolizing enzymes and DNA repair enzymes)¹².

From the analysis of several studies it was found that indeed there is a strong correlation between the exposure levels and exposure biomarkers; however, no evidence was

found regarding the styrene genotoxicity and therefore, it is necessary to conduct further studies on the use of susceptibility biomarkers to find genotoxicity evidence.

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Conflito de interesses

Os autores declaram não ter quaisquer conflitos de interesse.

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