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# Occupational exposure to styrene in the fibreglass reinforced plastic industry: comparison between two different manufacturing processes

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**Key words**
Styrene; fibreglass; biological and environmental monitoring

**Parole chiave**
Stirene; vetroresina; monitoraggio biologico e ambientale

**Summary**
**Background:** *Styrene is used in manufacturing fiberglass reinforced plastics: and occupational exposure was related to neurotoxicology and genotoxicity. The sum of the metabolites mandelic and phenylglyoxylic acids is the ACGIH biomarker for occupational exposure with a BEI of 400 mg/g of creatinine in end shift urine corresponding to an airborne styrene concentration of 85 mg/m$^3$. There are two main molding processes, open and closed, the last more effective at controlling worker's styrene exposure.* **Objectives:** *To compare the open molding process to the compression of fiber reinforced resin foils , a kind of closed molding, monitoring the styrene exposure of workers in two production sites (A and B).* **Methods:** *Environmental Monitoring was carried out by Radiello® samplers and Biological Monitoring by means of the determination of MA and PGA with HPLC/MS/MS in 10 workers at Site A and 14 at Site B.* **Results:** *The median values for styrene exposure resulted 31.1 mg/m$^3$ for Site A and 24.4 mg/m$^3$ for Site B, while the medians for the sum of the two metabolites in the end shift urine were 86.7 e 33.8 mg/g creatinine respectively. There is a significant linear correlation between personal styrene exposure and the excretion of styrene metabolites(R=0.74).* **Conclusions:** *As expected the exposure markers of the workers of the two production sites resulted higher in the open process. The analytical results of both environmental and biological monitoring were all below the occupational exposure limits, confirming the efficacy of the protective devices.*

**Riassunto**
***«Esposizione professionale a stirene in industrie di vetroresina: confronto tra due diversi processi produttivi».***
**Introduzione:** *Lo stirene è utilizzato nella manifattura plastici rinforzati con fibre di vetro e l'esposizione lavorativa è stata associata con neurotossicità e genotossicità. La somma delle concentrazioni di acido mandelico e fenil-*

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*gliossilico è l'indicatore biologico ACGIH per l'esposizione lavorativa a stirene, con un BEI di 400 mg/g di creatinina nell'urina di fine turno corrispondente ad 85 mg/m³ di stirene aereodisperso. Esistono due tipi di stampaggio, uno "aperto" e l'altro "chiuso", più efficace nel ridurre l'esposizione dei lavoratori.* **Obiettivi:** *Confrontare il processo aperto con quello di compressione di fogli di resina pronti, assimilabile al processo chiuso, monitorando l'esposizione a stirene dei lavoratori in due aziende (A e B).* **Metodi:** *Il monitoraggio ambientale è stato condotto con campionatori Radiello® e quello biologico mediante determinazione di acido mandelico e fenilgliossilico con HPLC/MS/MS su 10 lavoratori dell'azienda A e 14 dell'azienda B.* **Risultati:** *I valori mediani per l'esposizione a stirene sono risultati 31,1 mg/m³ per il sito A e 24,4 mg/m³ per il sito B, mentre le mediane della somma dei metaboliti nell'urina di fine turno sono risultate rispettivamente 86,7 e 33,8 mg/g creatinina. La correlazione fra i livelli di esposizione personale a stirene e l'escrezione dei metaboliti urinari è risultata statisticamente significativa (R=0,74).* **Conclusioni:** *Come atteso, gli indicatori di esposizione dei lavoratori delle due aziende sono risultati maggiori nel processo aperto. I risultati del monitoraggio ambientale e biologico sono tutti inferiori ai valori limite, confermando l'efficacia dei dispositivi di protezione.*

## Introduction

Fibre-reinforced plastics, also known as polymer composites, are successfully used in many industrial, aerospace, automobile and military applications. Examples include bathtubs and shower stalls, hulls for recreational and commercial watercraft, power tools, autovehicle parts, structural components for chemical process equipment and corrosion resistant storage tanks.

Styrene, due to its double vinyl-bond, has great chemical reactivity so it is widely used in the manufacture of polymers, resins, and reinforced plastics. Routes of occupational exposure can be both via inhalation (17) and, even if less important, via skin exposure (6, 8).

The major metabolic pathway of styrene in humans is the formation of styrene-7,8-oxide which is further metabolized by hydrolysis to styrene glycol, and then oxidized to mandelic (MA) and phenylglyoxylic (PGA) acids, whose total concentration is the dose biomarker suggested by the ACGIH for occupational exposure, with a BEI of 400 mg/g of creatinine in end-of-shift urine, corresponding to a TLV, in the case of environmental monitoring for a period of 8 hours, of 85 mg/m$^3$ equivalent to 20 ppm; non-occupational exposure to styrene is uncommon, but MA and PGA are considered non-specific biomarkers as they are metabolic products of other chemicals (1).

Trends in occupational exposure to styrene in the European Fibreglass-Reinforced Plastics (FRP) industry showed that the average styrene environmental concentration near open moulding workers decreased in the period 1966-1990 (by 5.3%) and then by 0.4% after 1990, with better conditions in North European industries compared to Southern Europe; similar data were collected on urinary mandelic acid as exposure biomarker (20). Nevertheless the evaporation of styrene from unsaturated polyester resin into the working environment during processing in the FRP industry can still produce significant workers' exposure to styrene (15).

Various publications and reports describe possible adverse health effects among workers in the industry (20); chronic exposure, in particular to low doses, has been associated with neurotoxic effects, slow reaction time, hearing deficits and mood disorders (14, 2); with chronic exposure nephrotoxic and hepatotoxic effects may also occur (3). Styrene exposure was reported to cause an increase in DNA and hemoglobin adducts and in the frequency of chromosomal aberrations (19); there is less evidence for an association between styrene exposure and the frequency of sister chromatid exchanges (16).

There are two general classes of composites manufacturing processes: open moulding and closed moulding. With open moulding, the gel coat and

laminate are exposed to the atmosphere during the manufacturing process; the most common open moulding process involves 'layup' of several layers, either by hand or some type of mechanical application equipment, such as a spray gun (at high or low pressure); in open mould fabrication, styrene evaporation occurs during the application of gel-coat or resin materials and continues until the gel- coat film or resin laminate has been cured (13). In closed moulding, the composite is processed in a two-sided mould set, or within a vacuum bag; the literature shows that closed moulding is very effective in controlling workers' exposure to styrene, reducing the environmental styrene concentrations by one order of magnitude (10).

The present paper explores the results of the environmental and biological monitoring of workers in two different manufacturing sites, one where the open moulding process was used, and the other where compression moulding of ready-to-use fibre-reinforced resin foils was performed, a process that can be considered a kind of closed moulding.

## Method

### Description of the studied enterprises

The two different manufacturing sites involved in the study were both located in Central Italy. We will call them site A and site B. Investigations were carried out in May at Site A (external temperature range 9-24°C) and in July at Site B (18-33°C).

Site A produced fibreglass-reinforced plastic containers and pieces to be used in different products like boats, furniture parts, or coatings and so on. In this enterprise workers used the hand lay-up open moulding process.

The manufacturing cycle of the fiberglass was characterized by the following phases:

1. manufacturing of moulds produced by stretching polyvinyl alcohol and wax on the surface of wooden models, to facilitate the detachment of the article when the forming operation is completed;
2. sheets of glass fibre were placed on the surface of the mould on which the resin is spread using machines or impregnating by hand using brushes;
3. the piece is then brought into a drying compartment, where the curing phase was completed. Once the product is dry and hardened, it is extracted from the mould;
4. the finishing phase included cutting, grinding and shaping of the parts to be assembled with other components for bonding (in the modelling compartment). The piece was then brought into the paint compartment for the final stages of manufacture.

The resins are products based on polyesters suspended in styrene, and the gel-coats are plastic paints based on unsaturated polyesters suspended in styrene; the glass fibres are the reinforcing material.

Production was located in one large area divided in three sections assigned to different tasks, but with no physical barriers between them: the first was used for model making, the second for the application of resins (figure 1) and reinforcing materials and the third for finishing the piece. The application of the resins and gel-coats took place in a special area equipped with a suction system with filters to capture most of the solvents and dust produced during this manufacturing phase. The personal protection equipment consisted of Tyvek® overalls, gloves and dust masks.

**Figure 1** - Hand lay-up of styrene resin of a medium-sized piece at site A. The worker is wearing gloves and a dust mask. The small bag at the waist is a pump for air sampling (data not reported here).

Site B produced fiberglass-reinforced plastic pieces to be used in different commercial activities (both small sized such as boxes for electrical supplies, and larger sized). The working area was a large shed in which every phase of the manufacturing process took place, and all the workers worked in this shed.

Compression moulding is a high-pressure method suitable for moulding fibreglass-reinforced plastic parts on a rapid time cycle. The mould set is mounted in a moulding press and the moulds are heated to 2500 to 4000 F. The moulding compound is supplied in large foils, protected by two plastic sheets (figure 2).

The manufacturing cycle can be thus described:

1. A charge of moulding compound is placed in the open mould.
2. The two halves of the mould are closed and pressure is applied. Depending on thickness, size, and shape of the part, curing cycles range from less than a minute to about five minutes.
3. The press is opened (figure 3) and the hot mould is extracted and left to cool.
4. The pieces are extracted from the moulds and finished.
5. Packaging and storage.

The parts made were typically used for: motor vehicles, construction, furniture and electrical components. The personal protection equipment consisted of gloves (also for heat protection), glasses and dust masks.

**Figure 2** - Foils of moulding compound as delivered by the supplier at site B

**Figure 3** - A press just opened – Site B

## Study subjects

An informed consent was used to clarify to the workers the objectives of the study and to determine the number of volunteers. A questionnaire was also administered to the enrolled workers in order to collect information on their present and past physiological and pathological history, dietary, alcohol and smoking habits, use of drugs, particularly enquiring about their occupational history, especially to gather information on any possible source of exposure to styrene both inside and outside the workplace. Questions were asked about the materials handled and the protection equipment used.

The study group at Site A consisted of 10 workers, 7 of which were females and all assigned to the hand lay-up task, and 3 males assigned to producing wood models and finishing the end products. The work shift started at 8.00 am and finished at 5

pm. Personal air sampling and urine sampling before the beginning and at the end of the work-shift were performed at Site A on the same day.

The study group at Site B consisted of 14 workers, all males, assigned to various tasks, working on three different shifts covering 24 hours (6 am - 2 pm.; 2 pm - 10 pm; 10 pm - 6 am); personal air and biological monitoring were carried out for all workers, covering the three different consecutive shifts in 24 hours: personal air sampling covered an 8h working shift and urine samples were collected before the beginning and at the end of each single work shift. In table 1 a description of the sample is presented, regarding age, gender, work task, smoking habits and alcohol consumption.

28 controls were recruited among volunteers, living in the same geographical area of the workers and not occupationally exposed to styrene, with age and gender as close as possible to the workers group: 12 for site A and 16 for site B, called C1 to C28, listed in table 1. They were requested to provide only one spot sample of urine and to fill in a short questionnaire, asking for details about smoking habits, alcohol consumption and job performed.

**Table 1** - Sample description: workers and controls

| | Site A | Site B |
|---|---|---|
| **Workers** | 10 | 14 |
| Age (SD) | 40.13 (6.17) | 41.50 (8.51) |
| Male | 3 | 14 |
| Female | 7 | 0 |
| Smoking | | |
| yes | 3 | 9 |
| no | 7 | 5 |
| Alcohol | | |
| yes | 8 | 11 |
| no | 2 | 3 |
| Task | | |
| Pressing/moulding | 7 | 12 |
| Finishing/carpentry/cutting | 3 | 2 |
| **Controls** | 12 | 16 |
| Age (SD) | 41.45 (8.47) | 39.07 (8.15) |
| Male | 6 | 12 |
| Female | 6 | 4 |
| Smoking | | |
| yes | 2 | 8 |
| no | 10 | 8 |
| Alcohol | | |
| yes | 10 | 15 |
| no | 2 | 1 |

## Environmental monitoring methods

Both environmental and personal monitoring of airborne exposure to styrene were performed using diffusive samplers (Radiello FSM, Italy). For environmental monitoring, static indoor samplers were performed; moreover, outdoor samples were coupled to indoor ones to identify the influence of indoor sources. As to individual monitoring, subjects wore the sampler in the breathing zone during the work shift. The collected styrene was recovered from the absorption tube by means of solvent extraction (2 ml of $CS_2$ in an ultrasonic bath for 30 minutes), and analysed by gas chromatography coupled with mass spectrometry (GC/MS).

### *Chemicals and supplies*

Radiello passive air samplers were supplied by Supelco. All reagents were of high purity analytical grade. The analytical reference standard of styrene was purchased from Riedel-de Haën (Buchs, Switzerland), deuterated styrene (d8) from Isotec, Inc. (Miamisburg, OH, USA). Carbon disulfide was purchased from Sigma-Aldrich (Steinheim, Germany). A Milli-Q water purification system (Milli-pore, Bedford, MA, USA) was used to supply high purity de-ionized water. A polyethylene glycol capillary column DB-WAXetr 123-7334 (30 m × 0.32 mm i.d., 1.00 μm film thickness; J&W-California, USA) was used for the chromatographic separation. Pure Helium (purity level 99.999%) was used as GC carrier gas (Air Liquid, Milan, Italy).

### *Instrumental analysis*

Quantitative determinations of airborne styrene were performed by a gas chromatograph (6890N Agilent Technologies) coupled with a single quadrupole mass spectrometer (5973 MSD System, Agilent Technologies). The peak areas were

integrated by the ChemStation Software (Agilent Technologies). The injection temperature (split ratio 1:10) was set at 230°C. Helium carrier gas flow rate in the analytical column was 1.5 mL/min. The column oven temperature was initially set at 50°C, then raised to 120°C with 2.5°C/min increments. Electron impact spectra were obtained with electron energy of 70 eV. Detection and source temperature were set at 150°C and 230°C, respectively. Detection was performed in the single-ion monitoring (SIM) mode. The mass-to-charge ratios (m/z) selected were 104 for styrene and 112 for styrene (d8), used as internal standard. Working calibration standard solutions, blanks and samples were analysed in triplicate and the average was used. The detection limit (LOD) and the lower limit of quantification (LLOQ) were 0.7 μg/m$^3$ and 1.9 μg/m$^3$ respectively. The intra- and inter-assay precision of the method was below 10%.

**Biological Monitoring Methods**

The biological monitoring of styrene was based on the determination of the two urinary metabolites, MA and PGA, as biomarkers of internal dose, using a HPLC/MS/MS method.

*Chemicals and supplies*

The analytical reference standards of DL mandelic acid and phenylglyoxylic acid were purchased from Fluka (Sigma-Aldrich, Germany). The deuterium labelled internal standards ± mandelic-D5 acid 99.4% and sodium phenyl-D5- glyoxylate 99.8% were obtained from CDN Isotopes (Quebec, Canada). Glacial acetic acid (100% Merck, Darmstadt, Germany), formic acid (50% Sigma-Aldrich, Germany) and purified water from a Milli-Q Plus system (Millipore, Milford, MA, USA) were used for preparing the mobile phase and for diluting the samples. Anotop 10 LC syringe filters (0.2μm pore size, 10 mm diameter) were purchased from Whatman Inc. (Maidstone, UK). A Kinetex 2.6 μ C-18 100 Å chromatographic column (100x 4.6 mm) was supplied by Phenomenex (USA) and used throughout the study. Urine samples were collected in plastic urine containers and immediately transferred into PTFE 50 ml screw cap tubes stored at –20°C until analysis.

*Instrumental analysis*

Each 10 ml urine sample was diluted with 10 ml of 2% acetic acid, spiked with 100 μl of internal standards (MA-D5 and PGA-D5) solution (10 mg/l), filtered on 0.2 μm syringe filter; 20 μl were injected into the HPLC-MS/MS system (API 4000, AB Sciex) for MA and PGA quantitative analysis.

Chromatographic separation was performed on a Series 200 LC quaternary pump (Perkin Elmer, Norwalk, CT, USA) using a 100x4.6 mm length, 100 Å of particle size, Kinetex 2.6 μm C-18 analytical column. The elution was carried out using a gradient of acetonitrile (phase A) and formic acid 0.2% (phase B), at a flow rate of 500 μl/min. Total run time was 10 min. The retention time of MA and its internal standard (MA-D5) was around 4.5 min, while for PGA and its internal standard (PGA-D5) the retention time was 4.0 min.

Detection was carried out in the negative ion, multiple reaction monitoring (MRM) mode, and parameters were optimized for the analytes by the automated "Infusion Quantitative Optimization" procedure and subsequently refined by flow injection analysis (FIA) using the pure standards. The following m/z ion combinations (precursor → product) were monitored and the transitions were as follows: m/z -150.9/-107.3 for MA, m/z -148.8/-105.1 for PGA, while for internal standard MA-D5 was -155.9/-112.0 and for PGA-D5 -153.8/-126.0. MS/MS instrumental parameters were as follows: dwell time 500 ms, curtain gas (arbitrary unit) 30, gas 1 (arbitrary unit) 12, gas 2 (arbitrary unit) 2, collisionally activated dissociation (CAD) (arbitrary unit) 4, collision cell entrance potential (ΔV) -10, collision energy (ΔV) -12, for all transitions, while collision cell exit potential (ΔV) was -5 for MA and PGA, -7 for PGA-D5 and -9 for MA-D5, cluster-breaking orifice voltage (ΔV) was -40 for PGA-D5, -45 for MA and PGA, -50 for MA-D5.

Quantitative determination for MA and PGA was performed using the isotopic dilution method

that allows determination of urinary concentration compensating for the matrix effect. LOD was 0.02 mg/l for MA and 0.015 mg/l for PGA, and LOQ 0.075 and 0.040 mg/l respectively. Accuracy was always higher than 82% and variability lower than 11% for both analytes.

### Statistical analysis

For the statistical analysis of results SPSS® version 19.0 (IBM® Corporation, Armonk, NY, USA) was used. The first study of data was conducted to evaluate the possible normal distribution of the values, and due to the small number the Shapiro Wilk Test was used; this test compares two alternative estimators of variance $\sigma^2$, a non-parametric and a parametric one: the range for the test result is from 0 to 1, with values <0.05 we reject the hypothesis of normal distribution. Application to our data indicated a non-normal performance of the data in most of the cases, and therefore subsequent correlations were all conducted using non-parametric tests.

In particular the Mann-Whitney test was used, which is applicable in the case of two independent samples in order to verify if they belong to the same population or not. The assumption is that the two samples come from the same population: with significance <0.05 this hypothesis is rejected and the difference between the two samples is confirmed to exist.

The univariate linear regression of the MA+PGA versus the airborne styrene concentration was studied. The slope and the intercept of the best fitted line were estimated by means of a standard least squares method. A confidence interval for the best fitted linear regression parameters was also estimated corresponding to a 95% confidence level. Data were analyzed by means of R statistical software (R Foundation for Statistical Computing, Vienna, Austria.ISBN 3-900051-07-0,URL http://www.R-project.org).

## Results and discussion

Exposure monitoring was performed for all workers, in order to assess potential exposures for all tasks; the cumulative results obtained for the personal air and biological monitoring of all workers, for those involved in moulding and for other tasks (both at the beginning of the shift and at the end of shift) are reported in table 2 for both sites, together with the urinary dosage of styrene metabolites for the two groups of controls.

Three workers at site A and two at site B not involved in the moulding/pressing tasks showed the lowest exposure values, showing that working conditions not linked with moulding, even if in the same area, do not cause a significant exposure to styrene; therefore these five workers were not included in the comparison between the two processes.

The data reported in table 2 show that there was a statistically significant difference between the end-of-shift concentration of styrene metabolites of exposed workers and controls both for site A and for site B.

Comparison of the results of biological monitoring of workers performed at the beginning and at the end of the work shift, both for site A and for site B, showed a significant difference between urine concentration values. The difference between the control group and the before-shift values of workers was also statistically significant, indicating that metabolism is not able to completely eliminate the styrene absorbed dose during the rest period, specially for site A but also for site B. This is due to the long elimination half-life of mandelic acid, up to 40 hours (1), and to the high partition coefficient (61.3) between adipose tissue and blood for styrene (9); in fact a specific accumulation in this tissue was shown (7) and the fraction of styrene accumulated is slowly released resulting in a delayed formation and excretion of urinary metabolites (12).

The linear regression between environmental and biological monitoring results of all workers at end of the shift is reported in figure 4 for both sites, together with its 95% confidence interval. There was a significant linear correlation between personal airborne styrene exposure and the excretion of styrene metabolites (R= 0.74 ).

For subjects with metabolite levels higher than expected (however within the 95% of confidence

**Table 2** - Results of environmental and biological monitoring of workers and urinary dosage of controls

| Study subjects | Statistics | Styrene (Radiello®) mg/m$^3$ | MA+PGA mg/g creat Before shift | MA+PGA mg/g creat End shift |
|---|---|---|---|---|
| Site A | Mean | 30.53 | 49.92 | 73.58 |
| All subjects | SD | 23.73 | 34.47 | 50.41 |
| | Median | 31.06 | 49.8 | 86.7 |
| | Min-max | 0.38-72.31 | 1.3-93.3 | 3-132.3 |
| | count | 10 | 10 | 10 |
| Site A | Mean | 41.58 | 63.2 | 103.0 |
| pressing/moulding | SD | 18.73 | 25.6 | 21.0 |
| | Median | 33.01 | 74.7 | 104.6 |
| | Min-max | 20.34-72.31 | 20.4- 93.3 | 76.7-132.3 |
| | count | 7 | 7 | 7 |
| Site A | Mean | 4.72 | 3.45 | 4.9 |
| Other tasks | SD | 7.42 | 3.04 | 3.03 |
| | Median | 0.48 | 3.45 | 3.3 |
| | Min-max | 0.38-13.29 | 1.3-5.6 | 3-8.4 |
| | count | 3 | 3 | 3 |
| Site A | Mean | N.A. | 1.95 | N.A. |
| Controls | SD | | 4.70 | |
| (spot urine samples) | Median | | 0.52 | |
| | Min-max | | 0.05-16.76 | |
| | count | | 12 | |
| Site B | Mean | 26.03 | 12.02 | 69.21 |
| All subjects | SD | 26.66 | 9.33 | 74.19 |
| | Median | 24.42 | 9.78 | 33.84 |
| | Min-max | 2.24-104.17 | 1.44-33.6 | 1.42-218.42 |
| | count | 14 | 14 | 14 |
| Site B | Mean | 30.33 | 14.6 | 85.9 |
| pressing/molding | SD | 26.34 | 8.9 | 75.6 |
| | Median | 26.60 | 13.5 | 47.1 |
| | Min-max | 2.53-104.17 | 4.33-33.6 | 2.52-218.4 |
| | count | 12 | 12 | 12 |
| Site B | Mean | 2.34 | 1.65 | 6.44 |
| Other tasks | SD | 0.13 | 0.3 | 7.1 |
| | Median | 2.34 | 1.65 | 6.44 |
| | Min-max | 2.24-2.43 | 1.44-1.86 | 1.42-11.46 |
| | count | 2 | 2 | 2 |
| Site B | Mean | N.A. | 0.9 | N.A. |
| Controls | SD | | 0.8 | |
| (spot urine samples) | Median | | 0.6 | |
| | Min-max | | 0.3-3.3 | |
| | count | | 16 | |

N.A.=not assessed

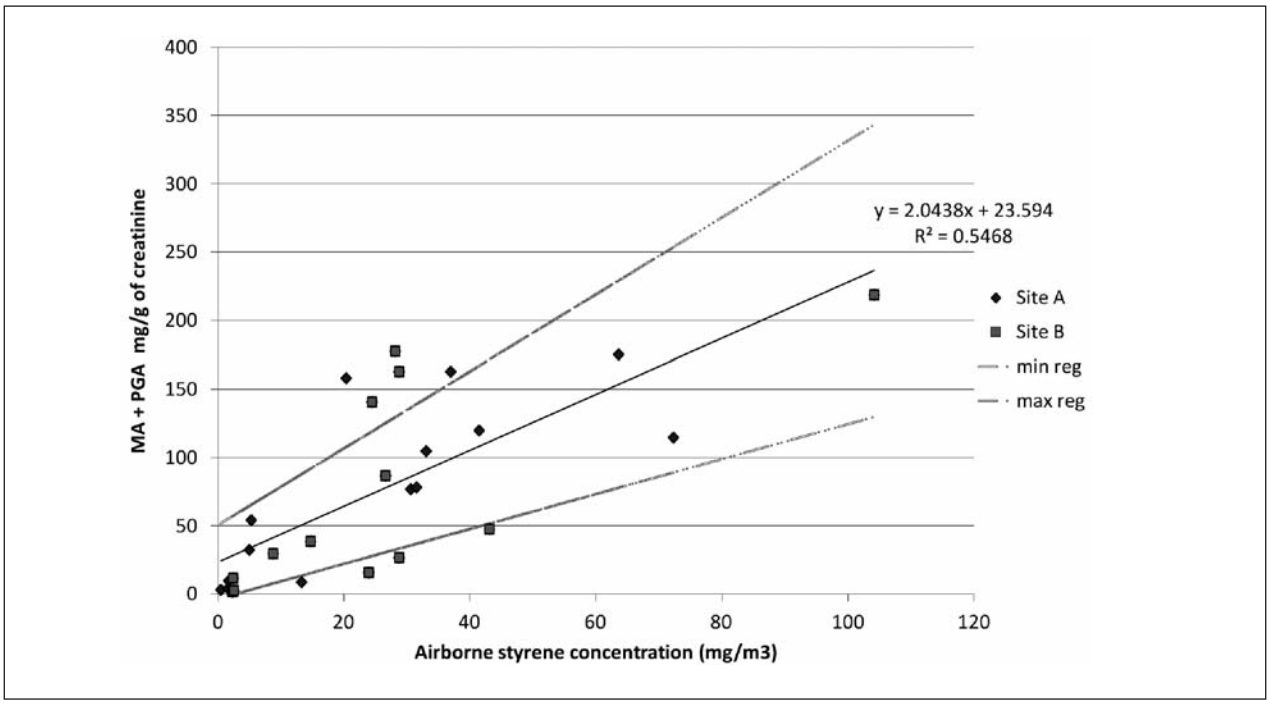


**Figure 4** - Correlation between environmental and biological monitoring. The dotted lines show the 95% confidence interval for the regression line

interval of the regression lines showed in figure 4) one possible hypothesis could be dermal absorption, even if a "skin" notation is not indicated for the latest ACGIH TLV of 20 ppm, while it was indicated for the 50 ppm TLV valid until 1996. The literature reports that "the percutaneous absorption of styrene is usually an insignificant exposure route and does not contribute significantly to the body burden of workers in the reinforced plastics industry" (11), while other literature data indicate instead that styrene damages human skin, even at concentrations comparable to the TLV, and that oxidative stress is involved in these effects (6). The workers examined wore gloves and dust masks with the main aim of protection against glass fibers, that are highly irritating, but specially at site B, some parts of the body (face, neck and in some cases arms) were not covered. Further studies need to clarify if climatic conditions characterized by high temperature and humidity, repeated exposures and damaged skin can increase styrene cutaneous absorption.

Unexpectedly, when results of Site B (compression moulding) are compared to those of Site A (open moulding), personal styrene exposures measured by Radiello® do not show statistically significant differences, nor do the concentration values of urinary metabolites at the end of the work shift.

However the values reported in table 2 (mean, median and minimum value) appear lower for Site B than for Site A, even if the difference is not statistically significant, probably due to the small size of the group, but data for site B are much more dispersed (standard deviation is higher). This could be interpreted as follows: even if the compression moulding process reduces styrene exposure of workers, the process at site B was not adequately under control and some subjects still presented very high exposure levels; this could be attributed to a lower risk perception leading to an incorrect use of personal protection equipment.

## Effect of smoking

Even if cigarette smoking is a known source of styrene (15, 4, 5), non-occupational exposure to styrene was defined as uncommon by the ACGIH (1). However, we compared the styrene biomarkers

levels of non- smoking controls (No.=16) to those of smoking controls (No.=8) and did not find any statistically significant difference (Mann Whitney, $p>0.05$). Therefore we considered cigarette smoking negligible with reference to the levels of mandelic and phenylglyoxylic acid in the urine measured by HPLC/MS/MS. Non-smoking controls were 17, but one result was considered an outlier (17.67 mg/g of creatinine for MA and 0 for PGA) and was not used for this comparison.

The values observed both for smokers and non-smokers were very much lower than the values measured on the workers and are comparable to the respective reference intervals estimated for MA and PGA in the general population in Italy: 0.084-2.339 and 0.009-1.238 mg/g creatinine, respectively (9).

## Conclusions

The study of two enterprises with different manufacturing cycles, the first using manual open moulding, the second compression moulding, did not produce the expected results, since the differences in the results of environmental and biological monitoring of the workers were not marked.

The difference between the MA and PGA urinary levels in the control group (that were comparable to the reference values for the general population in Italy) and the before-shift values of the workers confirms the slowness of styrene metabolism, while further studies are needed to clarify if high temperature and humidity, repeated exposures and damaged skin can increase styrene cutaneous absorption.

The compression moulding process should be able to reduce styrene exposure of workers with respect to open moulding, but some subjects at site B still presented very high exposure levels: this could be partly attributed to a lower risk perception leading to an incorrect use of protection devices.

Although analytical results were all below the occupational exposure limits (both environmental and biological), they show that styrene exposure could be further reduced, especially in compression molding, focusing attention on the relationship between risk perception and correct use of collective and personal protection devices.

No potential conflict of interest relevant to this article was reported

## References

1. American Conference of the Governmental Industrial Hygienists- ACGIH: TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices 2010, Cincinnati
2. Bergamaschi E, Smargiassi A, Mutti A, et al: Peripheral markers of cathecolaminergic dysfunction and symptoms of neurotoxicology among styrene exposed workers. Int Arch Occup Envir Health 1997; *69*: 209-214
3. Brodkin CA, Moon JD, Camp J et al: Serum hepatic biochemical activity in two populations of workers exposed to styrene. Occup Environ Med 2001; *58*: 95-102.
4. Chambers DM, Ocariz JM, McGuirk MF, Blount BC: Impact of cigarette smoking on volatile organic compound (VOC) blood levels in the U.S. population: NHANES 2003-2004. Environ Int 2011; *37*: 1321-1328
5. Charles SM, Batterman SA, Jia C: Composition and emissions of VOCs in main- and side-stream smoke of research cigarettes, Atmospheric Environment 2007; *41*: 5371-5384
6. Costa C, De Pasquale R, Silvari V, et al: In vitro evaluation of oxidative damage from organic solvents vapours on human skin. Toxicology in vitro 2006; *20*: 324-331
7. Engstrom K, Bjurstrom R Astrand I, et al: Uptake distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue. Scand J Environ Health 1978, *4*: 315-323
8. Eriksson K, Wiklund L: Dermal exposure to styrene in the fiberglass reinforced plastic industry. Ann Occup Hyg 2004; *48*: 203-208
9. Fiserowa-Bergerova V, Diaz ML: Determination and prediction of tissue/gas partition coefficients. Int Arch Occup Environ Health 1986; *58*: 75-87
10. Hammond D, Garcia A, Feng HA: Occupational exposure to styrene vapor in a manufacturing plant for fiber reinforced composite wind turbine blades. Ann Occup Hyg 2011; *55*: 591-600
11. Manini P, De Palma G, Andreoli R, et al: Determination of urinary styrene metabolites in the general Italian population by liquid chromatography–tandem mass

spectrometry. Int Arch Occup Environ Health 2004; *77*: 433-436
12. Minoia C, Perbellini L: Monitoraggio ambientale e biologico dell'esposizione professionale a xenobiotici. Vol. n. 6 *I Solventi*. Morgan edizioni Tecniche, 2001
13. Nunez CM, Bahner MA, Ramsey GH, et al: Pilot-scale evaluation of new resin application equipment for fiber reinforced plastics. Advances in Environ Res 2003; *7*: 301-310
14. Pahwa R, Kalra J: A Critical review of the neurotoxicology of styrene in humans. Vet Hum Toxicol 1993; *35*: 516-520
15. Papaleo B, Caporossi L, Bernardini F, et al: Exposure to styrene in fiberglass-reinforced plastic manifacture. Still a problem. JOEM 2011; *53 (11*): 1273-1278
16. Rueff J, Teixeira JP, Silva Santos L, Gaspar JF: Genetic effects and biotoxicity monitoring of occupational styrene exposure. Clinica Chimica Acta 2009; *399*: 8-23.
17. Stewart RD, Dodd HC, Baretta ED, Schaffer AW: Human exposure to styrene vapor. Arch Environ Health 1968; *16*: 656-662
18. Tang W, Hemm I, Eisenbrand G: Estimation of human exposure to styrene and ethylbenzene. Toxicol 2000; *144*: 39-50
19. Teixeira JP, Gaspar JF, Coelho P, et al: Cytogenetic and DNA damage on workers exposed to styrene. Mutagenesis 2010; *25*: 617-621
20. Van Rooij JGM, Kasper A, Triebig G, et al: Trends in Occupational Exposure to styrene in the European glass fiber reinforced plastics industry. Ann Occup Hyg 2008; *52*: 337-349