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Effects of concentration peaks on styrene neuroxicity in the fibreglass reinfoced plastics industry. Phase II

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Chemical Substances and Biological Agents

Studies and Research Projects

REPORT **R-728**

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Phase II

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SUMMARY

First, here is an analogy.

To properly explain the objective of this research project, we will first use an analogy. Suppose that two people of the same weight are invited to visit friends. At noon, their host opens a bottle of wine and offers some to them. The first person starts drinking very slowly so that by eight o'clock in the evening, he has consumed half of the bottle. The second individual starts drinking at seven thirty in the evening and therefore also drinks half a bottle, but all at once. Their host then proposes a game that involves walking a straight line with their eyes covered. The chances are great that the second person will have more difficulty walking a straight line.

The fibreglass reinforced plastics industry

In the fibreglass reinforced plastics industry (FRPI), the processes are generally discontinuous, meaning that the workers must apply resin containing various chemical substances over a large surface, but in a short period of time. This is what occurs in the manufacture of a boat hull made of fibreglass, or even a balcony platform. During a 10-, 15- or 30-minute period, the workers will therefore be exposed to rather high concentrations of products, including styrene. Then, sometimes for a few hours, the other operations will expose them only slightly to styrene. At the end of the day, if their average exposure is calculated, it will sometimes be determined that they were not extensively exposed (as in the case of the half-bottle of wine consumed over an eight-hour period) while some workers were exposed to high concentrations for a short period (as in the half-bottle consumed in 30 minutes).

The objectives of the study

The research project therefore had two main objectives. On the one hand, we wanted to perform continuous measurements in order to properly understand the exposure profiles of FRPI workers. On the other hand, we wanted to know whether "high" exposures for short periods of time, "peaks," can have an effect on the workers' nervous systems.

Measurements in the companies

In the framework of this study, we visited ten companies in the FRPI in the province of Québec. We recruited 104 subjects (51 men, 53 women) working in this industry. Using a gas chromatograph, we measured the styrene concentrations close to the workers' breathing zones over the entire day and, as a result, during the resin spreading operations on the surfaces of the parts to be coated. The volunteers were divided into three groups based on their average exposure concentration during the work shift. On average, the "Control group" was exposed to 7 mg/m^3, the "Average group" to 137 mg/m^3, and the "High group" to 333 mg/m^3. We also noted that there were in fact exposure "peaks." Subsequently, the volunteers were also classified according to their exposure or non-exposure to peaks: "Control group" (the same as previously), "Group without peaks" (not exposed to large variations in the concentration in the air), and "Group with peaks" (exposed to large variations in the concentration in the air).

Are the “peaks” toxic?

We subjected all the volunteers to a series of tests to verify whether there were slight modifications in their nervous systems. For example, we evaluated their capacity to differentiate very similar colours, their memory, their reaction time, and their attention (capacity to remain attentive throughout the duration of the test). We also measured the presence of certain symptoms such as nasal and throat irritation or discharge. To answer the question of styrene toxicity (average concentration and presence or absence of peaks), the groups of workers were compared on the basis of their results to the different tests.

Results and conclusion

To summarize the results of this project, we observed important styrene exposure peaks in the fibreglass reinforced plastics industry, even though in certain cases, the measured styrene values exceeded the standards prescribed by the *Regulation respecting occupational health and safety*. Furthermore, the volunteers exposed to styrene at average concentrations of 137 mg/m^3 showed a frequency of irritation symptoms higher than the “Control group” or the “Average group.” For the other tests and symptoms, the results showed no relationship between styrene, at the exposure levels in this study, and measurable effects on the nervous system. Also, the results obtained with the “Group with peaks” were no different from those obtained with the “Control group” and the “Group without peaks.”

It is important to note that in several studies, the styrene exposure duration (more than eight years) has been demonstrated to have a possible effect on the nervous system. This is an important factor to consider in studying the chronic effects of styrene. The average duration of exposure of the workers in our study was only 5.6 years, which could explain the negative results that we obtained. The concentrations of the peaks remain high in the FRPI and must continue to receive particular attention by occupational hygienists when measuring styrene concentrations in the workplace.

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1. INTRODUCTION

Styrene is an aromatic organic compound widely used in the manufacture of numerous polymers and copolymers such as polystyrene, acrylonitrile-butadiene-styrene, acrylonitrile-styrene, as well as styrene-butadiene latexes and rubbers. In the workplace, styrene exposure occurs mainly through the respiratory tract, and to a lesser degree through skin contact with the liquid form of this chemical. Skin absorption of styrene vapours is in fact negligible, except in extreme exposure situations such as reactor cleaning. The epidemiological study review carried out at the request of the Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST) furthermore showed that, in general, workers are exposed by inhalation to styrene vapours during the manufacture and use of plastic or resin (Vyskocil et al. 1997).

The question of revising the eight-hour time-weighted average exposure value (TWAEV) for subjects occupationally exposed to styrene is currently the subject of particular attention by regulatory organizations. The TWAEV in Québec is now 213 mg/m³ (50 ppm) and the short-term exposure value, the STEV, is 426 mg/m³ (100 ppm) (Éditeur officiel du Québec 2007). Critical analysis of the studies that have addressed styrene's toxic effects in exposed workers showed that central nervous system disorders and eye and upper respiratory tract irritation were most frequently reported (Vyskocil et al. 1997). In studies carried out in the fibreglass reinforced plastics industry (FRPI), where the workers' exposure was estimated by the time-weighted average concentrations, styrene-related neurotoxic effects have been identified. Since these effects are considered as being the critical effects, some authors are proposing the lowering of the TWAEV currently in force in Québec. In the last decade, researchers have also been interested in styrene-related ototoxic effects (Möller et al. 1990; Sass-Kortsak et al. 1995; Calabrese et al. 1996; Morata et al. 2002; Sliwinska-Kowalska et al. 2003; Sliwinska-Kowalska et al. 2005; Lawton et al. 2006; Triebig et al. 2009). While this association remains controversial for humans, studies conducted on rats show that styrene could induce cochlear lesions or even permanent hearing loss (Vyskocil et al. 1997).

The characterization of styrene exposure of workers in various FRP industries in Québec, conducted in the framework of Phase I of this project, demonstrated that styrene concentration peaks regularly occur in this industry. These peaks reach between three and six times the 8-hour TWA concentration and their duration is often between two and 17 minutes (Vyskocil et al. 2002). Based on these results, it appeared that the minimum atmospheric concentration for which styrene-related neurotoxic effects would be unlikely cannot be established by considering only the 8-hour TWA concentration. This could in fact lead to an incorrect estimation of the toxicological risk associated with workers' exposure to styrene.

2. REITERATION OF THE PROBLEM AND THE STATE OF KNOWLEDGE

In the critical analysis of the literature on the relationship between occupational styrene exposure and health effects, carried out at the IRSST's request (Vyskocil et al. 1997), we prepared a chart to estimate the reliability of each study. The items evaluated involved: *i)* the quality of the presentation of the subjects' styrene exposure, and *ii)* the quality of the evaluation of the adverse effects, including the control of confounding factors. From these two partial scores (points *i)* and *ii)* above), we proposed an overall reliability score for each of the studies. Later, this classification would allow a threshold concentration to be proposed, below which each of the reported neurotoxic effects would be unlikely.

With this preliminary study, we were able to highlight several points. First, the studies carried out on workers occupationally exposed to styrene vapours suggest that neurotoxic effects are probably the most sensitive indicators of styrene toxicity. However, the available data did not allow a NOAEL (no observed adverse effect level) and/or a LOAEL (lowest observed adverse effect level) to be established, whether it was following acute or chronic inhalation exposures. This literature review shows that neurotoxic effects are documented; however, the methodology of the studies does not allow the dose-effect relationship to be accurately established.

Furthermore, in several studies, the exposure was poorly documented, expressed in the form of average concentrations measured on the day of sampling. No indication was therefore supplied about the exposure peaks, which can contribute substantially to induced acute effects, or about long-term exposures, which produce chronic effects. In several studies, the urinary level of metabolites was used as bioindicator of the styrene exposure, even though the atmospheric concentration in the workplace often exceeded 640 mg/m³. At this exposure level, since the relationship between the urinary concentration of mandelic acid and the concentration of styrene in the inhaled air is not linear (Lof et al. 1993), it becomes impossible to link quantitatively the reported effects to the styrene concentrations measured in the air. Finally, two statistical limitations were noted. They involve the lack of power, due to the small number of studied subjects, particularly after the creation of sub-groups, and the lack of control of potential confounding factors. In fact, the workers could be concurrently exposed to other solvents or to other chemical compounds.

The earliest indicators of neurotoxic effects revealed in this review were the typical symptoms of central nervous system depression. A certain number of signs and symptoms, such as headache, fatigue, dizziness, nausea, changes in mood, discomfort, decreased arousal, etc., have been linked to this exposure. For the purposes of the study, we took into consideration the existence of concentration peaks and their possible impact on the expression of styrene toxicity. We report here the results collected from the literature review for each of the neurotoxic effects studied (Vyskocil et al. 1997).

In studies on volunteers, the diversity of the tests and exposure procedures used did not allow a consistent profile of styrene's acute effects to be developed. However, the number of reported symptoms seemed to increase when the acute exposure exceeded 426 mg/m³ styrene. For the studies carried out on workers, only the study by Triebig et al. (1989) provided precise indications about the lack of prenarcotic symptoms at styrene concentrations below 426 mg/m³.

But the actual threshold could well be below this value if the results of the studies of Edling et al. (1993) and Sassine et al. (1996) are taken into account, whose reliability had been classified as average; these studies suggested the presence of symptoms when the concentrations reached or exceeded 213 mg/m³ for 15 minutes. But this threshold could well be lower. The data from the studies carried out by Flodin et al. (1989) and Mergler et al. (1992), whose reliability was estimated as low, also corroborate these last results.

Among the indicators of styrene effects on the CNS, the "Simple reaction time" test was used in numerous studies, and the various authors generally observed a positive association between an increase in styrene exposure and an increased reaction time. In the study carried out by Kjellberg et al. (1979), this effect was even reported at an average time-weighted concentration as low as 37 mg/m³. From these results and taking into consideration the concentrations of the peaks, these data suggested with high reliability that styrene produces an increase in reaction time (acute effect) when the exposure concentration peaks reach or exceed values between 120 and 240 mg/m³ for 15 minutes. Moreover, since the results obtained by Mackay and Kelman (1986) and by Götell et al. (1972) showed that the effects on the reaction time occurred at concentrations much higher than 240 mg/m³, this value was retained as being the styrene exposure level above which an effect on the reaction time could not be rejected.

Furthermore, several studies provide evidence that styrene could induce an early loss of colour perception whose severity depends on the exposure dose. Based on the results obtained in the study by Chia et al. (1994), whose reliability was assessed as high, the establishment of a peak concentration threshold at approximately 240 mg/m³ for 15 minutes, beyond which colour perception difficulties would be detected, seems realistic. As well, workers with prolonged exposure would present a reduction in colour perception at even lower exposure levels.

For the studies that involved batteries of neurobehavioural tests to evaluate the neurotoxic effect on workers exposed to styrene, inconsistencies were identified. While two studies revealed acute effects (Schoenhuber et al. 1989; Letz et al. 1990) and one study showed chronic effects (Lindstrom et al. 1976), the study by Triebig et al. (1989) reported no neurotoxic effect related to styrene exposure. Also, in this last study, while the styrene concentrations reached 1069 mg/m³ for average exposure, and 2556 mg/m³ for the peaks, the scores obtained by the exposed subjects were not statistically significantly different from those obtained by the controls, for a battery of 12 tests.

The studies that reported an association between styrene exposure and nervous system alteration either had major deficiencies or identified only a few anomalies. As a consequence, it is impossible to determine, with high reliability, a concentration threshold at which neurobehavioural effects are observed. With the study of Mutti et al. (1984) as a basis, and taking into consideration the possible contribution of the peaks, the threshold concentration above which the test results would be changed would be above 300 mg/m³ over a period of 15 minutes.

In the framework of the critical literature review, the studies that investigated the effect of styrene exposure on nerve conduction velocity had major deficiencies. Evidence shows, however, that long-term exposure to styrene concentrations not exceeding 300 mg/m³ for 15 minutes cannot cause adverse effects on nerve conduction velocity (Vyskocil et al. 1997). Due to

the deficiencies identified in four studies in which the authors had recorded abnormal EEGs in workers exposed to less than 213 mg/m³ styrene, these results could not be retained (Vyskocil et al. 1997). In fact, these data did not allow the reported anomalies to be attributed to styrene exposure alone, and even less to the establishment of concentration thresholds below which this effect would be very unlikely. The neurochemical changes induced in styrene-exposed workers have also been the subject of several studies, but the contradictory results that were reported did not allow a clear conclusion to be drawn.

Analysis of publications on the styrene-exposure profiles of workers in the FRPI, the environment in which almost all of the studies were carried out (Vyskocil et al. 1997), allowed us to confirm our hypothesis regarding the occurrence of concentration peaks. In this industry, styrene concentrations in fact vary considerably over time for the same sampling site and depend on the manufacturing procedures. For example, during lamination, when the resins are applied manually or by spraying in open moulds, the concentration peaks generally reach three to six times the TWA concentration (eight hours) for all the TWA concentrations reported (37–714 mg/m³). Some recent studies, carried out in different countries, have reported exposure peaks lasting from 15 to 30 minutes, which suggests that it is a common phenomenon in this type of industry (Vyskocil et al. 1997). Some toxicokinetic studies have furthermore shown that the increase in styrene concentration in inhaled air associated with the peaks induces an increase in the blood concentration of this contaminant. The latter rapidly reaches equilibrium and the duration of this episode corresponds to the duration of the peaks (Truchon et al. 2003).

Our interest in studying the effects of exposure to styrene concentration peaks was the subject of an article published by Pierce et al. (1998). On four volunteers, these authors studied the relationship between styrene exposure (according to four profiles, two of which mainly involved concentration peaks) and some neurotoxic effects. The volunteers' exposure, estimated from the styrene concentration in the blood, was between 21 and 852 mg/m³ over a four-hour period. No effect was observed. This study however had two major drawbacks. The first involved the insufficient number of subjects (two to three for each experiment), and the second, the limited number of neurological tests used, which was only two. Interindividual differences for styrene metabolism and toxicity are well known; it is therefore impossible to dismiss the fact that the volunteers were simply less sensitive to the effects of styrene. Moreover, the use of only two neurological tests cannot cover all the aspects of neurotoxicity that can be attributed to this compound. Therefore, based on these data, a clear conclusion cannot be drawn about styrene's neurotoxicity. Nonetheless, by using toxicokinetic modeling, which made it possible to simulate the concentrations of this compound in the brain, the authors identified correlations between the inhaled-styrene concentration peaks or the styrene concentration peaks in the brain and the neurotoxic effects (Pierce et al. 1998).

In Phase I of this project, the exposure profiles of workers in ten Québec plants were evaluated (Vyskocil et al. 2002). The results confirmed the previous conclusions, i.e., the presence of concentration peaks. Defining a peak as a "point" concentration value reaching more than twice the average eight-hour concentration, significant concentration peaks were measured in eight companies. The peaks were as high as 16 times the TWAEV of 213 mg/m³, or even eight times the STEV of 426 mg/m³. Overall, the duration of the peaks varied between 2 and 17 minutes with, for the majority of the peaks, a variation between two and five minutes.

During Phase I, we also studied the acute effects on volunteers with a battery of tests, widely used globally, for evaluating the neurotoxicity of certain chemical substances (Vyskocil et al. 2002). Forty-two men were subjected to five styrene exposure scenarios for six hours: three stable concentrations (5, 106 and 213 mg/m³) and two variable concentrations (an average concentration equal to 106 mg/m³ with peaks reaching 213 mg/m³, and an average concentration equal to 213 mg/m³ with peaks reaching 426 mg/m³). Three categories of tests were used: sensory tests, neuropsychological tests, and subjective evaluation questionnaires. The results showed that exposure for six hours to concentrations not exceeding the TWAEV and the STEV in force in Québec has no impact on neurosensory performances, cognitive capacities, variations in mood aspects, or even the appearance of symptoms. Nonetheless, these results may have been influenced by several factors. First, the subjects had never been exposed to styrene before this study and the exposure time was short, only six hours. Also, the subjects were resting, under conditions of low pulmonary ventilation, which results in a lower absorbed dose and is not representative of the reality of workers in industry.

More recently, Viaene et al. (2001) focused on the relationship between styrene exposure and the appearance of neurobehavioural effects or their persistence in workers in a boat industry that had begun its activities in 1982. In 1989, for economic reasons, this industry stopped production and retained only some maintenance departments. Worker recruitment was done between the months of November 1992 and March 1993. The Exposed group consisted, on the one hand, of 90 people who were no longer exposed to styrene since the stopping of production, namely three years (Previously exposed group) and, on the other hand, of 27 people who were still exposed to this compound but to a lesser extent in the context of a new workstation (Currently exposed group). All these workers were matched with unexposed subjects from the Control group ($n = 60$). The workers' styrene exposure was estimated at 148 mg/m³ for the "Currently exposed group" and 157 mg/m³ for the "Previously exposed group."

For the period preceding the stoppage of production, the two exposed groups ("Previously exposed group" and "Currently exposed group") more frequently reported symptoms (irritation, headache, alcohol intolerance, concentration difficulties, reduction in smell, and nutritional imbalance) than the Control group. After adjustment for potential confounding factors (multiple linear regression), Viaene et al. (2001) observed that the number of symptoms increased significantly with the increase in the workers' average exposure concentration ($p = 0.03$). Furthermore, compared to the Control group, the "Previously exposed group" as well as the "Currently exposed group" obtained scores statistically significantly lower for the "Symbol digit matching" and "Digit span memory" tests ($p = 0.01$ and $p < 0.01$ respectively). For the score obtained for the "Hand-eye coordination test," only the "Previously exposed group" had lower scores than those obtained by the Control group ($p < 0.01$). For the three tests, the lower scores meant a change in performance. Viaene et al. (2001) observed no difference between the groups for the "Simple reaction time" and "Colour-word stress (response time to stimuli)" tests and for two tests evaluating memory ("Associated learning" and "Associated recall"). However, the increase in exposure duration was significantly associated with a lower arousal in the "Colour-word stress (response time to stimuli)" test. Viaene et al. (2001) concluded that exposure to average concentrations of styrene in the order of 155 mg/m³ over ten years or more may induce persistent neurotoxic effects.

By doing a meta-analysis that pooled the data of five studies, Benignus et al. (2005) concluded that an average styrene exposure of 86 mg/m³ over an eight-year period would induce an increase in the reaction time by approximately 6.5%. Furthermore, by comparing the effect of styrene exposure to that of age on the CCI, these authors estimated that a styrene exposure of 86 mg/m³ for eight years would induce an additional deficit equivalent to that acquired by an increase in age of 1.7 years.

Toppila et al. (2006) matched laminators (exposed) with non-laminators (controls) based on age in an FRPI. The TWA concentration of styrene was in the order of 108 mg/m³ for the exposed subjects and was below 20 mg/m³ for the controls. While postural stability remained constant with age for the non-laminators, Toppila et al. (2006) observed problems for this effect indicator that worsened with age for laminators. Nonetheless, better postural stability was measured for exposed individuals over 50 years of age compared to those from 40 to 49 years of age. The authors recognized that the data from their study did not allow this observation to be attributed to a lower styrene exposure level in individuals over 50 years of age.

Furthermore, it is important to note that in the FRPI, apart from styrene, acetone is used in large quantities for tool cleaning. Since acetone is a neurotoxic agent when present at high concentrations, it was important to take into account its possible contribution to the observed neurotoxic effects. For now, no study on the toxicodynamic interactions between styrene and acetone on the CNS has been found in the literature. According to Gong et al. (2002), at average concentrations of approximately 119 mg/m³ acetone (TWAEV of 1190 mg/m³), this compound has a negligible effect.

In this context, with the deficiencies and limitations of the documented studies, it became important to carry out a study on workers by characterizing the actual styrene exposure profiles and by using sensitive tests that make it possible to evaluate the neurotoxicity attributed to this contaminant.

3. OBJECTIVES

3.1 General objective

To contribute to the analysis of the toxicological health risk resulting from styrene exposure.

3.2 Specific objectives

1) To verify whether styrene's neurotoxic effects are linked to high styrene concentration peaks in workers exposed even to average concentrations not exceeding the TWAEV of 213 mg/m³;

2) To estimate workers' styrene exposure that can be associated with neurotoxic effects, based on the measurement of urinary mandelic acid, and to predict, using a toxicokinetic model, the styrene concentrations in the brain that will be produced as a function of the styrene exposure profiles that will be recorded in the industries;

3) To formulate a recommendation regarding the establishment of the standard for styrene and the interpretation to be made of the values of the TWAEV or STEV for styrene-exposed workers, in contexts where important exposure peaks occur.

4. METHOD

4.1 Recruitment of participating establishments

A preliminary evaluation of the industrial processes revealed that processes involving a polyester resin dissolved in styrene, and reinforced plastics, were best adapted to the needs of this project. In several plants in Québec, these processes require the manual intervention of "laminators," by far the most common and most exposed job in this industry. A list of establishments in the FRPI sector in Québec was created from two different sources. The first, the National Pollutant Release Inventory (NPRI), encompasses the establishments that use more than ten metric tonnes of a substance regulated by the NPRI, such as styrene, and that have the obligation of submitting an annual report to Environment Canada (Environment Canada 2009). The second source was the Web site of the Centre de recherche industriel du Québec ICRIQ (Centre de recherche industrielle du Québec "CRIQ" 2009). In a second step, this list was shortened according to the following criteria: the quantities of styrene used, the manufacture of fibreglass objects, the number of potentially exposed workers, a certain interest demonstrated during the first telephone exchange, or participation in Phase I of the study. To facilitate the recruitment of establishments suitable for the study, the support of the *Regroupement des industries des composites du Québec* (RICQ) was solicited. The industries that make up this group comprise more than 80% of the workers in the fibreglass industry in Québec and the great majority of large companies. Details of this process are presented in Appendix I.

4.2 Determining the number of subjects studied

In the grant application for Phase I, three statistical analysis protocols were used to determine the number of subjects studied: *i)* a protocol involving two-by-two and multiple comparisons between a reference group and an exposed group, *ii)* a one-way ANOVA protocol, and *iii)* a fixed ANOVA protocol.

A statistical parameter for evaluating the number of subjects required for such a study is the amplitude of the expected effect defined as follows:

$ES = \frac{\mu_1 - \mu_2}{\sigma}$ where μ_i is the average of the studied parameter in group i and σ is their common standard deviation. Generally, an ES below 0.25 is considered as corresponding to an effect of small amplitude. The smaller ES is, the larger the required size of the sample to reveal a difference between the groups.

We noted that regardless of the statistical analysis protocol considered, reasonable sample sizes in the order of 20 to 40 people correspond to:

1) Expected amplitudes of the effect varying between 0.10 (n = 40) and 0.14 (n = 20) with a statistical power of 80%, or between 0.11 (n = 40) and 0.155 (n = 20) with a statistical power of 90% when multiple two-by-two comparisons are done;

2) Expected amplitudes of the effect varying between 0.247 (n = 40), 0.287 (n = 30) and 0.354 (n = 20) for the one-way ANOVA protocol (with a power of 80%);

3) Expected amplitudes of the effect varying between 0.225 and 0.25 for the fixed ANOVA protocol (with powers of 80% and 90% respectively).

Based on this analysis, the decision was made to create groups of 40 subjects since this size should reveal the low amplitude effect expected in this study. It should be noted that this sample size exceeds the number of subjects examined in most of the studies that reported effects in prenarcotic symptoms or in the neuropsychological tests used in this study.

4.3 Recruitment of volunteers

Worker recruitment was done in fibreglass reinforced plastics industries in the province of Québec. To eliminate the "healthy worker effect," which can lead to an underestimation of the risk, particular attention was paid to the selection of an equivalent number of controls and exposed individuals in the same company. The Control group thus consisted of workers who performed different jobs within the company and were slightly or not exposed to styrene.

To participate in the study, the workers had: *i)* to be under 60 years of age; *ii)* to have been exposed to styrene for at least the last six months; *iii)* to not have a history of non-occupational exposure to neurotoxic agents, and *iv)* to not wear respiratory protection during the work shift. Workers with a history of brain pathology, diabetes or other endocrine diseases, kidney or liver diseases, cancer, or drug use were excluded. Workers with an alcohol consumption higher than the equivalent of 200 grams per week (fewer than 15 alcoholic drinks per week) were also excluded (Mergler et al. 1988; Campagna et al. 1996).

Workers exposed to solvents other than styrene, except for acetone, were also excluded. In fact, as was previously mentioned, on the one hand, no study has revealed an interaction between exposure to styrene and to acetone for effects on the CNS. On the other hand, since the average acetone exposure concentrations remain low in this type of industry, the effect of this compound is generally considered negligible (Gong et al. 2002). The workers' occupational solvent exposure history was also explored. Those individuals who had been exposed to solvents other than styrene during their professional activity before being employees in the FRPI were also excluded from the project. The purpose of this criterion was to eliminate confusion between the effects attributed to styrene and those that may be related to other solvents.

The information on the workers (age, education, medical history and occupational exposures) was evaluated from self-administered questionnaires (Appendix II; Appendix III). The medical history was reviewed by Dr. Alice Turcot (Direction régionale de la santé publique, Lévis). The protocol for this study was approved by the Comité d'éthique de la recherche (research ethics

committee) of the medical faculty of the Université de Montréal. The project was presented to the workers in each company during a meeting organized in the workplace, to which they were invited. The workers received three forms: Questionnaire I relating to medical history (Appendix II), Questionnaire II relating to exposure history (Appendix III), and a consent form (Appendix IV). The workers had at least one week to indicate their decision. The individuals solicited were completely free to participate in the study and even to withdraw at any time during the project. Furthermore, they received compensation of $100 for their participation. All the information gathered was handled anonymously and was used only for the purposes of this study.

4.4 Evaluation of exposure

4.4.1 Actual exposure

A. Average exposure during the work shift

For each of the workers, exposed or unexposed, the average styrene and acetone exposures during the entire work shift were measured for three consecutive days (Tuesday, Wednesday and Thursday) with the passive dosimetry method (3M #3500 organic vapour passive air monitoring badge). The analyses were performed at the IRSST using the gas chromatography method (IRSST method # 318-1).

B. Exposure profiles

Exposure profiles were obtained for the workers potentially exposed to concentration peaks, with the majority being laminators. Exposure to these peaks was characterized for one day out of the three work days, thus allowing the worker's exposure to be measured during his/her various tasks throughout the work shift. Two portable gas chromatographs (PGC), a Varian CP 2003 and a CP 4900, were used. These measuring instruments were equipped with a 10-m CP-Sil-5CB column and TCD (Thermal Conductivity Detector). A device to supply air to the PGC was developed to make up for a lack of power of the pump of the PGC probe. Briefly, a Gilair® type air sampling pump drew air continuously and during sampling with the PGC; it drew air from the connector junction. This installation had the effect of considerably reducing the dead volume of the probe, and as a result, the PGC's response time, and of minimizing inter-sample contamination (Appendix V). Furthermore, since styrene could adsorb onto the internal walls of the probe, Chemfluor® 367 (Thuot et al. 2004) was chosen from among three formulations of plastics verified for this use. During sampling, the probe was placed in the worker's breathing zone in order to have an exact measurement of the styrene concentration in the inhaled air. The use of two portable gas chromatographs allowed two exposed subjects to be sampled per day. The various tasks performed by the targeted worker as well as the time of their occurrence were noted, using a Dictaphone, at the same time as exposure characterization by PGC. Several photographs were also taken. This part of the protocol will provide invaluable indications to companies wanting to reduce the workers' occupational exposure to concentration peaks.

In the context of this project, the short-term atmospheric concentrations (measured with the PGC) that exceeded at least twice the TWA concentration were considered concentration peaks. This decision was motivated by the fact that the ratio of styrene's STEV over its TWAEV is equal to two. Furthermore, to be considered as having a profile with peaks, the worker not only had to be exposed to peaks (at least twice the TWA concentration), but had to have at the same time a minimum exposure to these peaks equivalent to 12.5% of the duration of his work shift. This percentage was established by referring to the *Regulation respecting occupational health and safety* (ROHS, 2007) which stipulates that the average concentration weighted over 15 minutes must not exceed the STEV more than four times during the work day, which corresponds to one-eighth in terms of the normal duration of an eight-hour work shift. Later, in order to differentiate the effects that can be attributable to the TWA concentration from those that are due to the concentration peaks, these two exposure parameters were considered simultaneously.

It is important to mention that in this study, the workers' exposure was to be stratified into three groups: *i)* controls (exposure < 5 ppm (21.3 mg/m³)); *ii)* average exposure of 50 ppm (213 mg/m³) without peaks, and *iii)* average exposure of 50 ppm with peaks. However, since the workers' real exposure could not be measured before sampling, it had to be estimated *a priori*. In Phase I of the project, the workstations as well as the tasks that induce exposure to styrene concentration peaks in the FRPI had been identified. In general, these workstations are occupied by laminators. First, this made it possible to select and classify the workers in relation to their presumed exposure level.

C. Biological exposure monitoring

In order to compare our results to the work of several authors, the relationship between a styrene exposure bioindicator and neurotoxic effects was studied. Mandelic acid (MA), one of styrene's metabolites, was measured using the method developed by the IRSST's laboratories (IRSST method # 106-1) based on high performance liquid chromatography (HPLC). The urine sample at the end of the workday was collected on the day of the neuropsychological evaluation. For the exposed workers, this day corresponded mainly to the day of exposure sampling by PGC. The urine samples were stored at a temperature of 4°C and transported to the IRSST laboratory.

In conformity with the provisions relating to reportable poisonings or diseases, the *Regulation under the Public Health Act* of Québec stipulates that any mandelic acid result exceeding 3000 micromoles/litre of urine must be reported to the public health director. When this project was being developed, this provision did not exist. As a result, in the original protocol, this analysis was systematically done following an agreement supplied by the worker, but the mandatory nature of the reporting to the public health director that subsequently occurred did not allow compliance with the confidentiality commitment reached with the companies. When one of the company representatives showed apprehension regarding this confidentiality problem, the research protocol was changed. The company directors could therefore have the choice of accepting or refusing this type of sampling before submitting the request to the worker. A request for modification was proposed on July 4, 2004, to the IRSST and was accepted three months later. During this period, the study was suspended. Finally, biological samples were completely excluded from the project as of September 2005 following the events presented in 5.2.2.

4.4.2 Exposure history

In addition to the quantitative exposure evaluation carried out by measuring the ambient air and by biological monitoring, we proceeded with an evaluation of each worker's styrene and acetone exposure history from self-administered questionnaires subsequently validated by an occupational hygienist and existing data (Appendix III). This retrospective evaluation covered two periods: *i)* the period preceding the worker's current job, and *ii)* the period at the current job preceding the measurement campaign.

For the first period, or historical period, the worker's work history was collected in one section of the self-administered questionnaire. An initial list covers the jobs held and the dates (type of job, period of employment in months or years, name of the company). Next was a series of short open-ended questions on the use or the presence of a series of products. The products to be checked (no, yes) included the most important sources of neurotoxic products in the workplace (solvents, paints, gasoline, adhesives, certain welding operations). If the worker checked yes for a product, he had to indicate in which job(s) this product was found and what tasks were performed. This part of the questionnaire was evaluated by an expert hygienist before the final selection of the workers in order to eliminate *a priori* those who had previously been exposed to significant concentrations of neurotoxic products other than styrene or acetone.

A second part of the self-administered questionnaire dealt with the current job. The questions covered the description of the tasks performed at the current workstation, which determined whether past exposure could have been different by asking about the other jobs that could have been occupied in the past in this company. To make a judgement about previous exposure at the current job, the expert hygienist used the data present in the company's health records maintained at the CSSS for workstations similar to that of the worker at the time of his evaluation. These data are qualified as "historical" in the remainder of this report. The final analysis would indicate whether the exposure measured during the intervention was representative of the worker's past exposure.

4.4.3 Simulation by toxicokinetic modeling

A. Styrene concentration in the brain

The profiles of styrene concentrations in the brain were obtained by toxicokinetic modeling in a context of exposure or non-exposure to concentration peaks by inhalation. The physiologically based toxicokinetic model (PBTK) developed during Phase I of the project was used for this. This approach made it possible, first, to study the possible variations in styrene concentration in the brain associated with concentration peaks in the inhaled air. Then, by simulating these variations in brain concentration, their relationship to potential neurotoxic effects could be analyzed.

B. Urinary mandelic acid in the case of exposure to styrene alone

The workers' urinary excretion of mandelic acid as a function of the styrene concentrations in inhaled air was described with the PBTK model developed. The mandelic acid concentrations were first simulated for all the workers based on their respective levels of exposure or absence of exposure to peaks. The results of this simulation were then compared to the data obtained from 40 available urine samples.

C. Urinary mandelic acid in the case of simultaneous exposure to styrene and acetone

To study the impact of simultaneous exposure to styrene and acetone, present in the studied workplaces, on mandelic acid excretion, the modified toxicokinetic model (mixed model) was used. The data relevant to the development of a model for acetone had already been reported (Truchon et al. 2003). This approach had also been applied to other mixtures of solvents (Tardif et al. 1997; Ali et al. 1999). For each worker, mandelic acid excretion was simulated according to two exposure scenarios, namely exposure to styrene alone or the simultaneous exposure to styrene and acetone. The concentrations in the inhaled air measured for each of the two solvents were used, and the hypotheses of a competitive or non-competitive metabolic inhibition were tested. These two solvents are in fact metabolized by CYP2E1.

4.5 Neuropsychological evaluation of the effects of styrene

Styrene's effects on the nervous system have been evaluated using a battery of "Swedish Performance Evaluation System" tests (Gamberale 1989), used for evaluating the subjects' neurosensory and neuropsychological response.

Three categories of tests were used: subjective evaluation questionnaires, neuropsychological tests, and sensory tests. These tests are widely used globally and are recognized as being valid by organizations such as the World Health Organization (WHO) (1993) and the Swedish National Institute of Occupational Health (1996). Choice of these tests was motivated by their psychometric quality: *i)* sensitivity to early neurotoxic effects; *ii)* test-retest stability; *iii)* detailed measurements; *iv)* precise measurements; *v)* standardization of the guidelines and terms of execution; *vi)* reduced learning effect; *vii)* duration of the evaluation not exceeding 1.5 hours, and *viii)* control of the effect of fatigue.

The battery of tests initially described by Iregren et al. (1996) was developed on a DOS computer platform. An adaptation was done, followed by validation to allow operation on Windows.

4.5.1 Description of the tests

A. Self-evaluation questionnaire

The results of the self-evaluation questionnaire are entered in the computer. The questionnaire has two parts, the first on mood and the second on the presence of symptoms. In the first part, 12

adjectives describing mood are proposed. For each of the adjectives, the subject must identify the most appropriate description of his condition based on a scale of seven categories from “not at all” to “completely.” The results are expressed as scores that describe four aspects of mood (Arousal + ; Arousal – ; Stress + ; Stress –). For the second part, the evaluation consists of 17 symptoms (Appendix VI). The subject must answer by giving the intensity that he feels on a scale of seven, from “not at all” to “actually very strong.”

B. Neuropsychological tests

All the neuropsychological tests were presented on computer, thus allowing their standardized administration conditions to be respected. The following tests were proposed to each subject:

“Simple reaction time”:

The “Simple reaction time” test consists of pressing on the keyboard spacebar as rapidly as possible when a red square appears on the screen. The measurements obtained represent the average of the reaction times. The dominant hand is used.

”Colour-word stress” (response time to stimuli):

For the “Colour-word stress (response time to stimuli)” test, the names of four colours appear one at a time on the screen and can be in a colour other than what the word says. The subject must press on the keyboard spacebar only when the name of the colour corresponds to the colour of the letters (correct answer). A version testing the response inhibition capacity was used (75% of the stimuli are correct answers and the inter-stimuli interval is 1.5 seconds). Measurements for the inhibition test are the average reaction time for the correct answers and the percentage of correct answers. The dominant hand is used.

“Symbol digit matching”:

The “Symbol digit matching” test consists of presenting two two-line tables simultaneously on the screen. For the first table, the first line corresponds to a series of nine symbols associated randomly with numbers (from one to nine) illustrated on the second line. For the second table, only the first line consists of symbols. By referring to the first table, the subject must enter on the second line of the second table the numbers corresponding to the symbols appearing on the first line of the first table. The order of the symbols is different in the two tables. In total, ten rows are presented. The measurement obtained is the average reaction time.

“Digit span memory”:

For the “Digit span memory” test, a series of numbers is presented on the screen at one-second intervals. The subject is asked to reproduce on the keyboard the series of numbers. Depending on success or failure, the number of digits presented in the next series is increased or reduced. The score is defined as being the maximum length of the span and corresponds to the maximum number of digits correctly reported at least three times. It should be mentioned that the measurement is the direct recall of the series of digits.

“”Continuous tracking test” (Joystick tracking):

For the “Continuous tracking test” test, a large rectangle containing a small rectangle moves on the screen following a path unknown by the subject. The subject must keep the small rectangle as close as possible to the centre of the larger one. The score corresponds to the average deviation from the centre of the rectangle throughout the duration of the five-minute test.

C. Sensory tests

“Lanthony desaturated colour D-15” test:

The “Lanthony desaturated colour D-15” test evaluates colour discrimination capacity. The subject must place in order, according to colour resemblance, 15 coloured disks placed randomly in front of him. The test is performed under standard lighting (D75 – 7500K from X-Rite which acquired Amazys Holding AG, formerly GretagMacbeth) and the measurements are done monocularly. The test is of unlimited duration. The colour confusion index (CCI) without error has a value of one (Bowman 1982).

“Vistech VCTS 6000”:

The “Vistech VCTS 6000” test measures the sensitivity thresholds for uncoloured contrast vision. The worker is presented striped circles with alternate light and dark bands whose spatial frequency (1.5; 3; 6; 12 and 18 cycles/degree) and contrast (a scale of 9) are varied.

4.5.2 Evaluation process

The workers began the tests by answering the questionnaire on mood and symptoms. They then did the other tests. The sensory and neuropsychological tests were presented in an order determined by the Latin squares method so as to control for the effect of the presentation order. Each subject completed the tests in front of a computer (four parallel evaluations), under the supervision of an evaluator to ensure that the evaluation was carried out properly. Each volunteer had one opportunity to become familiar with the material prior to the evaluation for the purposes of this study.

4.6 Statistical analysis

Styrene’s effects on central nervous system function were evaluated by stratifying the workers’ exposure into three groups: Control group (< 42.6 mg/m^3; < 10 ppm), Average group (42.6 – < 213 mg/m^3; 10 – ≤ 50 ppm), and High group (> 213 mg/m^3; > 50 ppm). This stratification takes into account the TWAEV in force in Québec, which currently is 213 mg/m^3 (50 ppm). The results were analyzed by taking styrene exposure into consideration according to two scenarios: *i)* the weighted average on the day of the neuropsychological evaluation; and *ii)* the presence or absence of peaks. Three potential confounding factors were furthermore retained. They were age,

education level, and cigarette consumption. Since the possible effects of alcohol consumption had been eliminated by selecting workers whose weekly alcohol consumption was less than 200 g, no additional adjustment for this parameter was done. The degree of statistical significance retained for the results was 0.05.

Based on the nature of the dependent variable represented by the score obtained for the different tests, either a "one-way ANOVA" or the Kruskal Wallis test was used. Therefore, in the case of the four neuropsychological tests (Simple reaction time; Colour-word stress (response time to stimuli); Symbol digit matching; Continuous tracking test), comparison of the average of the scores obtained was done by a one-way ANOVA. For these tests, the calculated score in fact represents a continuous type variable. For the two other neuropsychological tests, namely the percentage of errors in the "Colour-word stress (response time to stimuli)" and the "Digit span memory" tests, since the "Score" dependent variable is ordinal in nature, comparison of the median of the distribution of the scores in the three groups was done with the Kruskal Wallis test. This test was mainly used for between-group comparison of the scores obtained for the visual tests ("Lanthony desaturated colour D-15; Vistech VCTS 6000") and for the distribution of the symptoms or distribution of the aspects of mood. For all of the analyses, when the obtained results supported the existence of statistically significant differences between the groups, a Post Hoc test in the case of the one-way ANOVA, or a multiple comparison procedure in the case of the Kruskal Wallis test, were used to identify the difference or differences.

The various results were presented graphically in an Outlier Box-Plot. The box is centered on the median and its lower and upper limits respectively represent the first and third quartiles of the distribution (Q_1 and Q_3 respectively). The lower and upper limits of the box plot indicate the minimum and maximum values of the distribution, namely $[Q_1 - 1.5 \times (Q_3 - Q_1)]$ and $[Q_3 + 1.5 \times (Q_3 - Q_1)]$. The confidence interval at 95% is represented in dark gray. Furthermore, since reference values for the various tests in a population of workers are not available in the literature, the distinction between extreme values and outliers was very uncertain. In this context, the scores exceeding the maximum and minimum values of the distribution ($[Q_1 - 1.5 \times (Q_3 - Q_1)]$ and $[Q_3 + 1.5 \times (Q_3 - Q_1)]$) were replaced by these limit values (winsorization) (Dixon 1960; Dixon et al. 1974). The purpose of this procedure was to minimize the impact of the extreme values without eliminating their contribution.

5. RESULTS

5.1 Recruitment of participating establishments

5.1.1 Creation of a list of establishments in Québec with workers potentially exposed to styrene

Table 1 summarizes this list produced according to the criteria presented in section 4.1.

Table 1: Search for establishments based on NAICS codes and types of products manufactured

Establishments		Number of workers	
Type	Number	Production	Other
Establishments that manufacture reinforced plastic products	143	4588	1113

5.1.2 Creation of a list of establishments suitable for recruitment

Based on the criteria presented in section 4.1, a list containing some fifty establishments was extracted from the list in Table 1.

5.2 Recruitment of workers

Based on the list established in 5.1.2, two consecutive processes were undertaken to recruit workers complying with the search criteria.

5.2.1 First worker recruitment process

The first recruitment process was undertaken through direct contact with the establishment by telephone or through the person in charge of the establishment (i.e., within the CSSS occupational health team). This process proved ineffective because it led to the recruitment of only two establishments with a total of five workers. It rapidly became apparent that the participation of the large companies in the sector was essential and that support from the milieu was necessary to obtain this participation (Appendix VII).

5.2.2 Second worker recruitment process

A. Obtaining the participation of the RICQ

The RICQ, which had given its support to Phase I of this study, had expressed its initial opposition to Phase II. A major effort was made to regain the participation of the RICQ in this study because the RICQ's board of directors is made up of representatives of the largest companies in this sector, and this association represents more than 80% of the workers in the manufacture of reinforced plastic products in Québec.

A presentation of the Phase II objectives and aspects was made to the RICQ on April 14, 2004. Its content is found in Appendix VIII. Basically, the RICQ's reservations about participating in Phase II of the study related to a poor interpretation of the STEV by certain members of its board of directors (BoD). Following this meeting, the RICQ's BoD recommended that its members participate in this study at a meeting of the members of the BoD held May 4, 2004 (Appendix IX).

B. Procedure with the establishments

Following the support of the RICQ, contacts could be established with companies in the sector, and participation agreements were thus obtained.

In the list of establishments suitable for recruitment, several establishments were eliminated because: i) they did not manufacture reinforced plastic products, ii) in the end, they were not interested in participating, iii) they did not have significant exposure to styrene, or iv) all the exposed workers wore respirators.

Recruitment had to be suspended from mid-July 2004 to mid-October 2004 while waiting for the IRSST to authorize resumption of the work following the problems associated with the regulation on reportable diseases. It was again suspended from the fall of 2005 to the spring of 2006; the RICQ suspended its support following the DSP's (public health department's) inappropriate disclosure of mandelic acid results to an establishment before the research team had informed the management of this establishment. Following this incident, new procedures were undertaken with the RICQ to again obtain its support. An agreement establishing the process for the remainder of the study was reached in May 2006 (Appendix X). Finally, some ten establishments in the FRPI in the province of Québec participated in the present study.

5.3 Results of the neurotoxic tests

5.3.1 Description of the studied population and exposure measurements

In total, 104 workers (51 men, 53 women) were recruited in the participating establishments. In this population, the average age was 38 years (range: 21–57 years) and the average duration of employment was 67 months, or 5.6 years (range: 3 months–31 years).

The studied population's exposure was stratified by taking into account several parameters. First, by using the time-weighted average concentration on the day of sampling, the workers were classified into three groups: Control group (< 42.6 mg/m³; < 10 ppm), Average group (42.6 – < 213 mg/m³; 10 – ≤ 50 ppm), and High group (> 213 mg/m³; > 50 ppm) (Table 2). The average styrene concentration (± standard deviation) to which the workers were exposed was 7.0 ± 7.7 mg/m³ (1.6 ± 1.8 ppm) in the Control group, 136 ± 51 mg/m³ (32 ± 12 ppm) in the Average group, and 332 ± 95mg/m³ (78 ± 22 ppm) in the High group. Table 2 presents the minimum, median and maximum values of the acetone and styrene concentrations measured in the study, as well as the fraction of the TWAEV corresponding to each of these values. It also presents the minimum, median and maximum ratios of the measured fraction of acetone over its TWAEV, and the measured fraction of styrene over its TWAEV. While styrene reaches 244% of its regulated limit value, the maximum concentration of acetone represents only 36% of its own TWAEV (Table 2).

Table 2: Average exposure values for styrene and acetone as well as their ratios with respect to the TWAEV

	STYRENE		ACETONE	
	Concentration in the air (mg/m^3)	% TWAEV	Concentration in the air (mg/m^3)	% TWAEV
MINIMUM	53	25	35	2.9
MEDIAN	205	96	155	13
MAXIMUM	520	244	430	36

In order to evaluate whether chronic effects for exposed workers in the fibreglass reinforced plastics industry can be associated with styrene concentration peaks, the exposure profile was mainly considered. The subjects studied were then classified according to their profile category obtained using the PGC: Control group (unexposed), Group without peaks (exposed without peaks), and Group with peaks (exposed with peaks) (Section 4.4.1, point b). The results of this study showed that among those exposed, 40 workers were classified "Without peaks" and 20 workers "With peaks." The average duration of the peaks was 7.0% of the duration of the work shift (Range = 0.00–12.3%) in the workers "Without peaks" and 15.2% (Range = 12.5–22.1%) in the workers "With peaks." Among the latter, 11 workers belonged to the Average group, and 9 to the High group. Moreover, to verify whether styrene-related neurological problems would be linked even more to the concentration peaks, caused by certain tasks, than to the TWA concentration, the "High group" was stratified into a "High group without peaks" and a "High group with peaks." The duration of the average historical exposure (± standard deviation) was 6.5 years (± 7.6 years) for the workers in the Average group, and 4.7 years (± 4.7 years) for those in the High group.

The average styrene exposure concentration during the work shift was calculated mainly from the point concentrations recorded during sampling with the PGC (average of the profile's point concentrations). For the exposed workers, a significant correlation was observed between the increase in the TWA concentration obtained by passive dosimetry and that of the average of the profile (R2 = 0.528; $p < 0.0001$) (Figure 1).

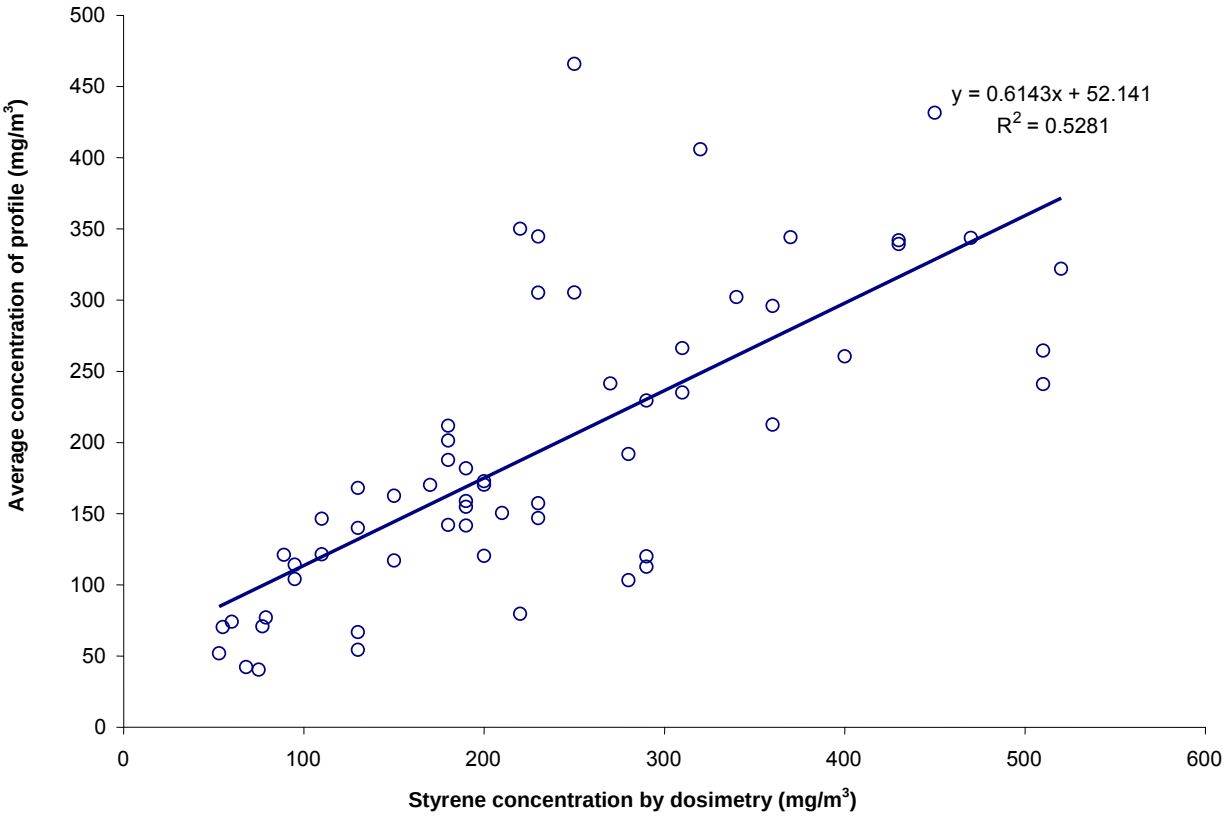

Figure 1: Relationship between the average styrene concentration during the work shift obtained from the point concentrations recorded during PGC sampling (average of profile) and the TWA concentration

In a later step, the historical styrene concentration for the studied workers was estimated. This evaluation could not be done in a rigorous way for three reasons. First, CLSC (local community service centre) records were not available for all of the companies, and second, when this information was available, the description of the tasks from the CLSC reports corresponded only partially to what was described during measurement. Also, in the visited establishments, information that could have allowed past exposure to be estimated from that measured during this study was not available. For example, this was the case for data on measurement of the manufacturing processes, production rates, or even the means implemented for controlling exposure. The duration of employment in this type of industry was on average 5.6 years.

Besides styrene exposure, the possible explanatory factors for the neuropsychological test scores were age and education level. Furthermore, since cigarette consumption may have an impact on the nervous system, this factor was also taken into consideration in the statistical analysis. Regardless of the styrene exposure measurement considered (TWA concentration and presence or absence of peaks), comparison of the distribution of the three potential confounding variables for the three groups did not reveal any statistically significant difference (Table 3 and Table 4). As indicated in the methodology (Section 4), the effects that could be styrene-related were then studied by comparing the distribution of the scores obtained for each test (dependent variable) for the three exposure groups (independent variable).

Table 3: Comparison of the distribution of the potential confounding factors for the three exposure groups

Factors	Exposure groups			p (ANOVA)
	Control (0 – 42.6)	Average group (42.6 – 213)	High group (> 213)	
Average age (SD), Years	40 (10) (n = 35)	37 (10) (n =31)	35 (10) (n = 31)	0.141 (n = 97)
Average education (SD) (Years)	11 (2) (n = 33)	11 (2) (n = 31)	10 (2) (n = 30)	0.119 (n = 94)
Average tobacco use (SD) Cigarettes/d	5 (8) (n = 35)	4 (8) (n = 31)	4 (6) (n = 31)	0.602 (n = 97)

Note: SD = standard deviation; n = number of subjects

Table 4: Comparison of the distribution of the potential confounding factors for the controls and the two exposure groups based on the presence of peaks

Factors	Exposure groups			p (ANOVA)
	Control	Exposed		
		Without peaks	With peaks	
Average age (SD), years	40 (10) (n = 35)	37 (10) (n =40)	36(9) (n = 20)	0.221 (n = 95)
Average education(SD), years	11 (2) (n = 33)	10 (2) (n = 40)	11 (2) (n = 19)	0.330 (n = 92)
Average tobacco use (SD), (Cigarettes/D)	5 (8) (n = 35)	3 (7) (n = 40)	6 (7) (n = 20)	0.205 (n = 95)

Note: SD = standard deviation; n = number of subjects

5.3.2 Results of the analysis of styrene neurotoxicity in relation to the TWA concentration

A. Self-evaluation questionnaire

1. Symptoms

The medians obtained for the symptom score in the three exposure groups as well as the results of the Kruskal Wallis test are presented in Table 5 and Appendix XI.

Table 5: Comparison of the median scores obtained for symptoms in the three exposure groups

Symptoms	Exposure groups (mg/m^3)			p (Kruskal Wallis) (n = 94)
	Control (0 – 42.6) (n = 35)	Average (42.6 – 213) (n = 30)	High (> 213) (n = 29)	
Headache	0	0	0	0.115
Dizziness	0	0	0	0.805
Nausea	0	0	0	0.588
Fatigue	2	2.5	3	0.010*
Chest pressure	0	0	0	0.029*
Cough	0	0	0	0.447
Shortness of breath	0	1	1	0.222
Eye irritation	0	0	1	0.028*
Eye discharge	0	0	1	0.027*
Blurred vision	0	0.5	1	0.389
Nasal irritation	0	0	2	0.024*
Nasal discharge	0	0.5	2	0.001*
Impression of unpleasant odours	0	1	2	0.000*
Throat irritation	0	0	2	0.028*
Impression of unpleasant taste	0	0	2	0.000*
Skin irritation	0	1	1	0.383
Vertigo	0	0	0	0.657

Note: * Significant ($p < 0.05$)

The data in Table 5 show that the median of the scores for the symptoms measured on a scale of seven does not exceed a score of 3. The results of the statistical analysis summarized in Table 5 show no significant difference between the exposure groups regarding the median of the scores obtained for each of the following symptoms: headache, dizziness, nausea, cough, shortness of breath, blurred vision, skin irritation and vertigo. However, these data highlight the significant difference between the median of the distribution of the symptoms of upper respiratory tract irritation in the High group (> 213 mg/m³) and those of the Average group (42–213 mg/m³) and Control group (< 42.6 mg/m³). These symptoms involve irritation with discharge from both the eyes and nose as well as throat irritation. Such a difference between the groups was also observed for two other symptoms, namely the impressions of chest pressure and unpleasant taste.

In the two styrene-exposed groups (Average group and High group), the median obtained for the state of fatigue and the impression of unpleasant odours was significantly different from that measured with the Control group. In addition, for the impression of unpleasant odours, a difference was also noted between the medians obtained for the High group and the Average group.

In considering the results observed for all of the symptoms, respiratory tract irritation is seen to be more frequently reported in the High group than in the Average group or Control group.

From these data, a threshold for upper respiratory tract irritation can be established for an average exposure in the order of 137 mg/m³. This exposure level in fact corresponds to the average obtained for the Average group.

2. Mood

The medians obtained for the mood aspect scores for the Controls and the two Exposed groups as well as the results obtained for the Kruskal Wallis test are presented in Table 6.

Table 6: Comparison of the median scores obtained for mood in the three exposure groups

Mood state	Exposure groups (mg/m³)			p (Kruskal Wallis) (n = 94)
	Control (0 – 42.6) (n = 35)	Average (42.6 – 213) (n = 30)	High (> 213) (n = 29)	
Arousal +	11	12	8	< 0.0002 *
Arousal –	6	4	6	0.101
Stress +	9	9	7	0.159
Stress –	4	4.5	5	0.665

Note: * Significant ($p < 0.05$)

The results of the statistical analysis performed with the Kruskal Wallis test for the various aspects of mood (Table 6) show that only the Arousal + aspect of mood showed a significant difference between the groups. In fact, the stress aspects (Stress + and Stress –) like the Arousal – mood aspect showed no statistically significant difference between the three exposure groups. The multiple comparison procedure determined that the High group, exposed to styrene concentrations above 213 mg/m^3, presents a median of the "Arousal + mood" index significantly statistically lower than that observed with the Control group (< 42.6 mg/m^3) or the Average group (42.6– < 213 mg/m^3). Based on this analysis, the Arousal + mood aspect appears to be lower in workers in the High group than in those in the Average group or Control group. From these data, it can be established that it is probable that the Arousal + aspect is affected by an average styrene exposure above 137 mg/m^3. This value in fact corresponds to the average exposure obtained with the Average group.

B. Neuropsychological tests

In general, for the neuropsychological tests (Simple reaction time; Colour-word stress (response time to stimuli); Symbol digit matching; Digit span memory; Continuous tracking), the results obtained demonstrate no statistically significant difference ($p < 0.05$) between the scores obtained in the three styrene-exposure groups.

1. "Simple reaction time"

For the "Simple reaction time," the average score was 0.324 seconds (95% CI = 0.301; 0.348 seconds) for the Control group, 0.328 seconds (95% CI = 0.307; 0.351 seconds) for the Average group, and 0.329 seconds (95% CI = 0.310; 0.348 seconds) for the High group. The one-way ANOVA showed no statistically significant difference ($p = 0.671$) between the average times obtained for the three groups. The distribution of the results appears in Figure 2.

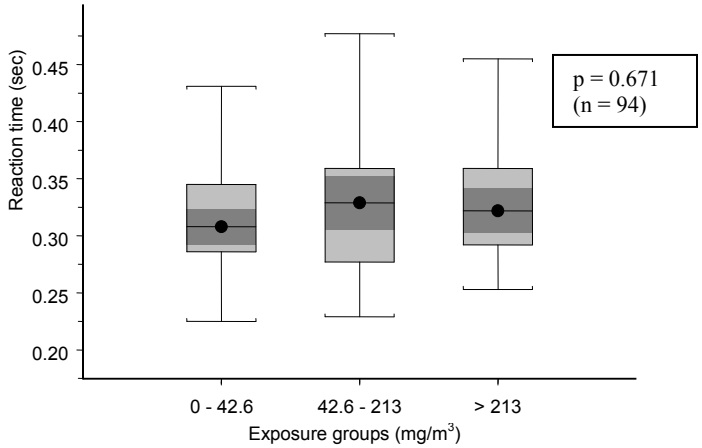

Figure 2: Comparison of the results obtained for the "Simple reaction time" test for the three styrene-exposure groups. Note: Box plot description available in section 4.6; the results are adjusted for age, education and cigarette consumption.

2. "Colour-word stress (response time to stimuli)"

For the "Colour-word stress (response time to stimuli)," the evaluations involved the average reaction time for correct answers, as well as the percentage of correct answers. For the reaction time, the average time was 0.579 seconds (95% CI = 0.561; 0.596) for the Control group, and 0.571 (95% CI = 0.558; 0.584) and 0.572 seconds (95% CI = 0.558; 0.585) for the Average group and High group, respectively. The one-way ANOVA revealed no statistically significant difference (p = 0.727) between the average times obtained for the three groups of workers. The distribution of the results appears in Figure 3.

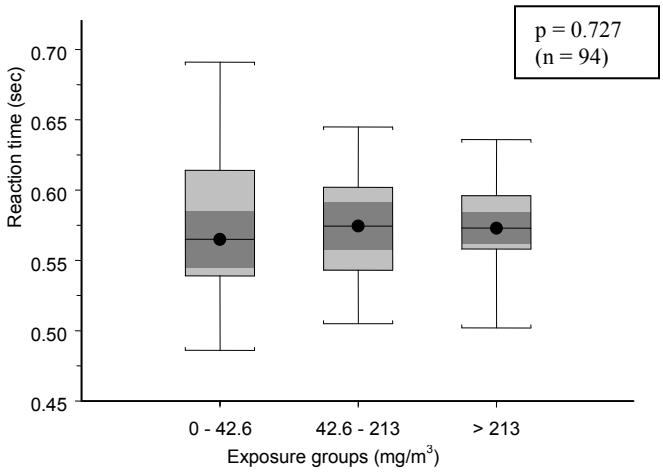

Figure 3: Comparison of the results obtained for the reaction time for the "Colour-word stress (response time to stimuli)" test for the three styrene-exposure groups. Note: *idem* Figure 2.

As with the reaction time, the score for the percentage of correct answers to the "Colour-word stress (response time to stimuli)" was not statistically significantly different for the three groups of participants (p = 0.766) (Figure 4). For this score, the calculated medians were in fact 0.955 (Interquartile = 0.854–0.980) for the Control group, and 0.953 (Interquartile = 0.917–0.980) and 0.948 (Interquartile = 0.907–0.965) respectively for the Average and High groups.

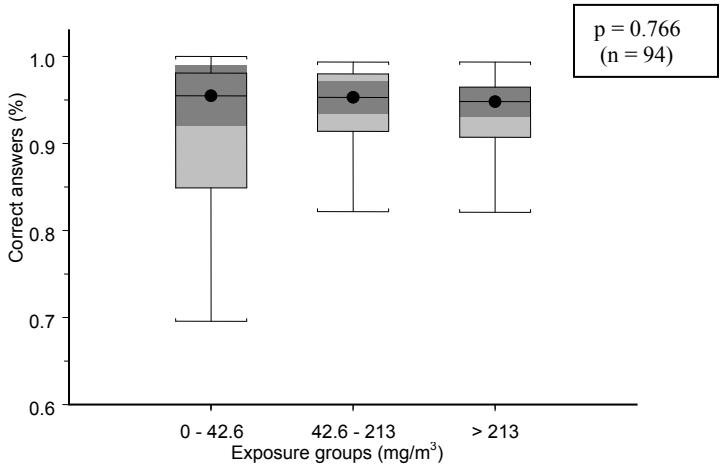

Figure 4: Comparison of the results obtained for the percentage of correct answers for the "Colour-word stress (response time to stimuli)" test for the three styrene-exposure groups. Note: *idem* Figure 2.

3. "Symbol digit matching"

For the "Symbol digit matching," in order to standardize the dependent variable, the score data were logarithmically transformed. The one-way ANOVA revealed no statistical difference between the averages of the scores obtained for the three groups of workers studied (Figure 5) ($p = 0.348$). The averages of the logarithms of the times observed were in fact 1.030 seconds (95% CI = 0.980; 1.080) for the Control group, 0.972 seconds (95% CI = 0.905; 1.040) for the Average group, and 1.008 seconds (95% CI = 0.949; 1.066) for the High group.

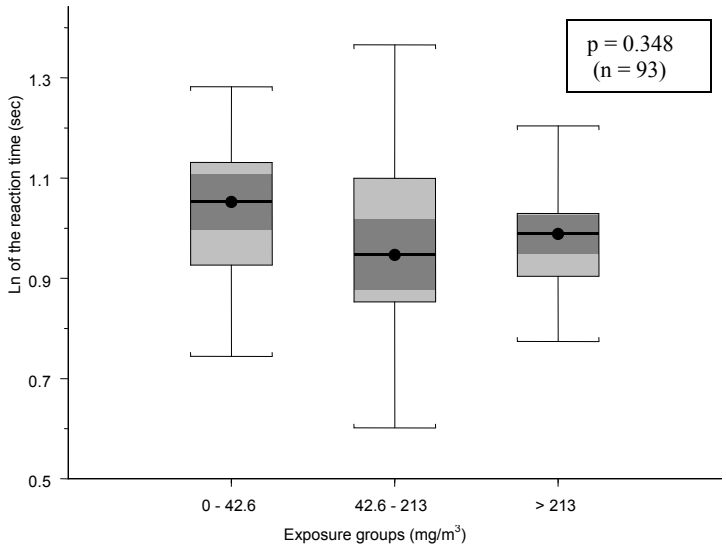

Figure 5: Comparison of the results obtained for the "Symbol digit matching" test for the three styrene-exposure groups. Note: *idem* Figure 2.

4. "Digit span memory"

For the "Digit span memory" test, the medians obtained for the three exposure groups were identical, namely 6 (Interquartile = 5–7), such that the analysis carried out with the Kruskal Wallis test revealed no statistically significant difference between these groups ($p = 0.293$) (Figure 6).

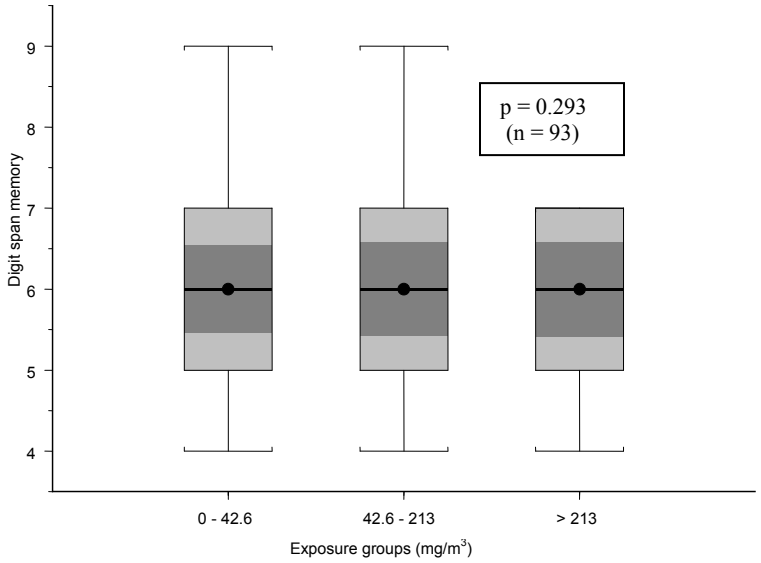

Figure 6: Comparison of the results obtained for the "Digit span memory" test for the three styrene-exposure groups. Note: *idem* Figure 2.

5. "Continuous tracking"

For the "Continuous tracking" test, the one-way ANOVA test revealed a difference between the average scores. Post-hoc analysis revealed a statistically significant difference between the High group and Average group ($p = 0.032$) (Figure 7). It should be noted that no difference was identified with the Control group. The averages of the observed scores were 0.041 (95% CI = 0.037; 0.044) for the Control group, 0.037 (95% CI = 0.034; 0.040) for the Average group, and 0.043 (95% CI = 0.040; 0.047) for the High group.

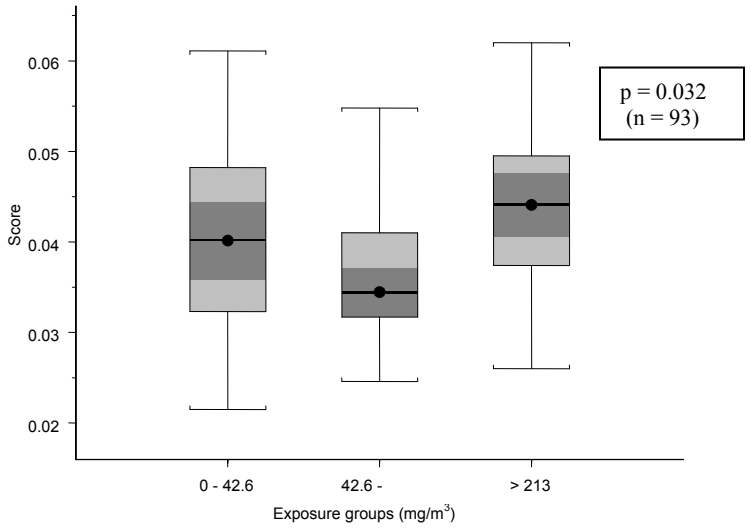

Figure 7: Comparison of the results obtained for the "Continuous tracking" test for the three styrene-exposure groups. Note: *idem* Figure 2.

At this stage in the analysis, the effect of dexterity in joystick use on the observed score was questioned. Besides the possible effect of confounding factors such as age, educational level, and smoking, which were similar between the groups, it seemed important to consider the gender factor. Study of this variable in the population sample showed that the proportion of women and men was respectively 51% and 49% in the Control group, 26 and 74% in the Average group, and 84% and 16% in the High group. This difference between the groups was furthermore statistically significant ($p < 0.0001$).

To analyze the association between styrene exposure and the "Continuous tracking" test score by simultaneously controlling for all of the confounding factors including gender, a multivariate analysis was carried out. This procedure revealed that only the coefficients of regression estimated for gender and age were statistically significant ($p = 0.000$). In fact, for the Average group (42.6–213 mg/m³) as for the High group (> 213 mg/m³), the degrees of significance of the estimated coefficient of regression were 0.65 and 0.50 respectively. For education level, the degree of significance of the estimated coefficient was at the limit of significance ($p = 0.07$). Based on this analysis, styrene exposure cannot be considered as a possible explanatory factor for the "Continuous tracking" test score.

In summary, the results of this study showed no statistically significant association between styrene exposure at the levels described and the scores obtained for the different neuropsychological tests used.

C. Sensory tests

1. "Lanthony desaturated colour D-15"

The "Lanthony desaturated colour D-15" test was used to study whether variations in the colour confusion index can be linked to styrene exposure. This test was done on both the right eye and left eye. For both eyes, the median obtained for this index in each of the groups is reported in Table 6. As mentioned in Section 4.5.1 (point c), a colour confusion index (CCI) (Bowman 1982) without error is 1.

Table 7: Summary of the results obtained for the colour confusion index for the "Lanthony desaturated colour D-15" test for the three exposure groups

	Exposure groups (mg/m^3)			**p (Kruskal Wallis)**
	Control (0 – 42.6)	**Average group (42.6 – 213)**	**High group (> 213)**	
CCI Right eye Median	1.125 (n = 34)	1.112 (n = 31)	1.294 (n = 29)	0.081 (n = 94)
CCI Left eye Median	1.139 (n = 35)	1.102 (n = 31)	1.207 (n = 29)	0.483 (n = 95)

Note: CCI = Colour confusion index

The results of the statistical analysis performed with the Kruskal Wallis test did not demonstrate a significantly different colour confusion index for the groups (Table 7; Figure 8; Figure 9). For the right eye and left eye, a lack of association between styrene exposure and the colour confusion index was noted (right eye, $p = 0.081$; left eye, $p = 0.483$).

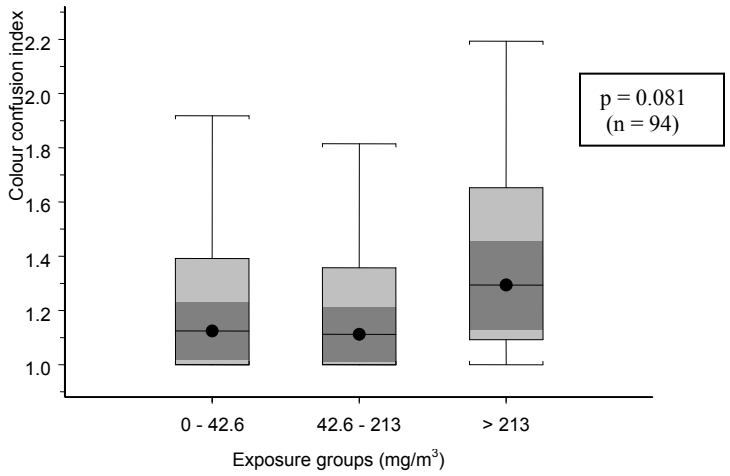

Figure 8: Comparison of the results obtained for the colour confusion index for the right eye for the "Lanthony desaturated colour D-15" test for the three styrene-exposure groups. Note: *idem* Figure 2.

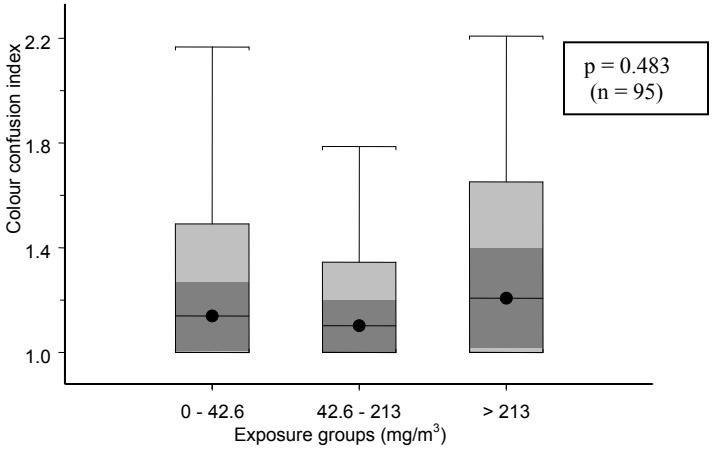

Figure 9: Comparison of the results obtained for the colour confusion index for the left eye for the "Lanthony desaturated colour D-15" test for the three styrene-exposure groups. Note: *idem* Figure 2.

2. "Vistech VCTS 6000"

The "Vistech VCTS 6000" visual test yielded the contrast sensitivity thresholds for the various frequencies for all of the workers. For each frequency, the distribution of thresholds was compared for the three exposure groups. The medians of these distributions as well as the results of the comparisons of the groups obtained with the Kruskal Wallis test are presented in Table 8. Analysis of the synthesized data in this table shows no significant difference in sensitivity to contrasts for the three exposure groups.

Table 8: Summary of the results obtained for contrast vision for the "Vistech VCTS 6000" test for the three exposure groups

Parameter measured	Frequencies (Cycles/degree)	Exposure groups (mg/m^3) Control (0 – 42.6)	Average (42.6 – 213)	High (> 213)	p (Kruskal Wallis)
Vision contrast threshold Median right eye	**1.5**	35	35	35	0.951 (n = 93)
	3	85	85	85	0.996 (n = 93)
	6	70	70	70	0.234 (n = 93)
	12	55	32	32	0.909 (n = 93)
	18	15	15	10	0.676 (n = 93)
Vision contrast threshold Median left eye	**1.5**	35	35	35	0.508 (n = 94)
	3	85	85	44	0.270 (n = 94)
	6	70	70	70	0.573 (n = 94)
	12	32	32	32	0.845 (n = 94)
	18	10	10	10	0.945 (n = 94)

As in the neuropsychological tests, the results of this study do not identify a significant statistical association between styrene exposure at the measured levels and a change in visual function as evaluated with the "Lanthony desaturated colour D-15" and "Vistech VCTS 6000" tests.

5.3.3 Results of the analysis of styrene neurotoxicity in relation to concentration peak exposure

5.3.3.1 Exposure to peaks without considering the TWA concentration

Styrene neurotoxicity associated with concentration peak exposure was analyzed by first considering the exposure profiles recorded by PGC. These results are presented in three parts: the self-evaluation questionnaire, the neuropsychological tests, and the sensory tests.

A. Self-evaluation questionnaire

1. Symptoms

The results of the comparison of the symptom scores for the three exposure groups according to profile category are presented in Table 9.

Table 9: Comparison of the median scores obtained for symptoms for the three exposure groups according to profile category

Symptoms	Exposure groups			p (Kruskal Wallis) (n = 94)
	Control (n = 19)	Exposed		
		Without peaks (n = 40)	With peaks (n = 35)	
Headache	0	0	0	0.859
Dizziness	0	0	0	0.697
Nausea	0	0	0	0.685
Fatigue	2	2.5	3	0.010*
Chest pressure	0	0	0	0.538
Cough	0	0	0	0.247
Shortness of breath	0	0.5	1	0.060
Eye irritation	0	1	1	0.194
Eye discharge	0	0	0	0.034*
Blurred vision	0	1	0	0.397
Nasal irritation	0	0	1	0.109
Nasal discharge	0	1	1	0.010*
Impression of unpleasant odours	0	1	2	0.001*
Throat irritation	0	0	2	0.118
Impression of unpleasant taste	0	1	2	0.004*
Skin irritation	0	0.5	1	0.274
Vertigo	0	0	0	0.075

Note: * Significant ($p < 0.05$)

The data summarized in Table 9 show that in general the median of the symptom scores is low and does not exceed the value of 2 on a scale of 7. In fact, only the fatigue score reaches 3 on this scale. Also, of all of the 17 symptoms studied, only five showed a median of the scores significantly different for the three exposure groups ($p < 0.05$). They are: fatigue, eye and nasal discharge, as well as the impression of unpleasant odours and unpleasant taste. The multiple comparison procedure revealed that the median of the distribution of four symptoms (fatigue, nasal discharge and impression of unpleasant odours and unpleasant taste) in the two exposed groups ("With peaks" and "Without peaks") was statistically significantly different from that obtained in the Control group. For the last symptom, eye discharge, while the Group without peaks had a median significantly different from that of the Control group, there was no difference noted in comparison with the Group with peaks. As Table 9 indicates, no significant difference was noted between the exposure groups for the median of the scores obtained for the

other symptoms, namely: headache, dizziness, nausea, chest pressure, cough, shortness of breath, eye irritation, blurred vision, nasal irritation, throat irritation, skin irritation and vertigo. Even though the median of the scores obtained for nasal, throat and skin irritation was higher in the Group with peaks than in the Group without peaks, this difference was statistically non-significant.

2. Mood

The results of the comparison of the scores for the mood states for the three exposure groups according to the profile category are presented in Table 10.

Table 10: Comparison of the median scores obtained for mood for the three exposure groups according to profile category

State of mood	Exposure groups			p (Kruskal Wallis) (n = 94)
	Control (n = 19)	Exposed		
		Without peaks (n = 40)	With peaks (n = 40)	
Arousal +	11	9.5	9	0.099
Arousal –	6	5	5	0.485
Stress +	9	9	7	0.347
Stress –	4	5	5	0.711

From the analysis of the data summarized in Table 10, a lack of association emerges between styrene exposure, described according to profile category, and the scores obtained for the mood state.

In summary, the results of the present study do not establish a statistically significant association between exposure to styrene concentration peaks and the increase in frequency of symptoms or the variation in mood states in the sample of workers studied. In general, based on the statistical criteria established, the presence or absence of association between this exposure and the median of the score obtained for the symptoms was no different for the two profile categories of the Exposed group (“Without peaks” and “With peaks”). Mainly, it is impossible to reject a certain effect of the peaks based solely on the data obtained for eye discharge.

B. Neuropsychological tests

For the neuropsychological tests, as with styrene exposure expressed in terms of TWA concentration, comparison of the means or medians obtained for the exposure groups according to profile category (Control group; Group with peaks; Group without peaks) showed no significant difference for the tests ("Simple reaction time," $p = 0.577$; "Colour-word stress (response time to stimuli)," $p = 0.642$; "Percentage of errors for colour-word stress," $p = 0.852$; "Symbol digit matching," $p = 0.133$; "Digit span memory," $p = 0.816$; "Continuous tracking," $p = 0.338$). Figures 10 to 15 illustrate these results.

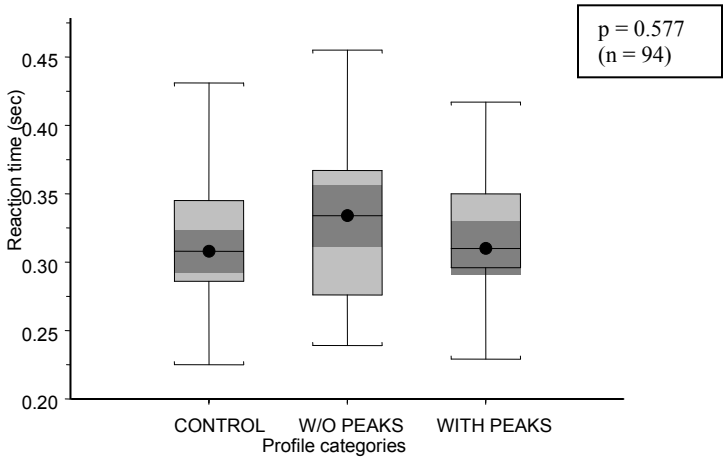

Figure 10: Comparison of the results obtained for the "Simple reaction time" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

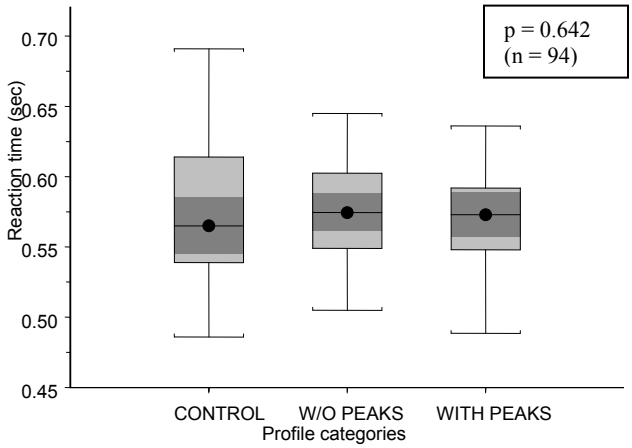

Figure 11: Comparison of the results obtained for the reaction time for the "Colour-word stress (response time to stimuli)" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

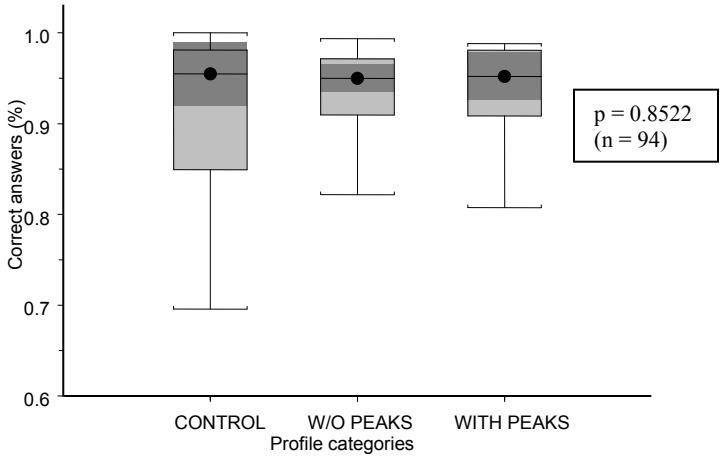

Figure 12: Comparison of the results obtained for the percentage of correct answers for the "Colour-word stress (response time to stimuli)" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

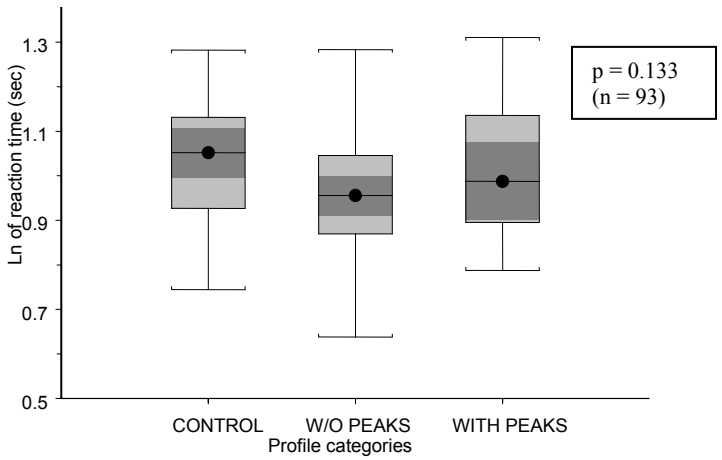

Figure 13: Comparison of the results obtained for the "Symbol digit matching" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

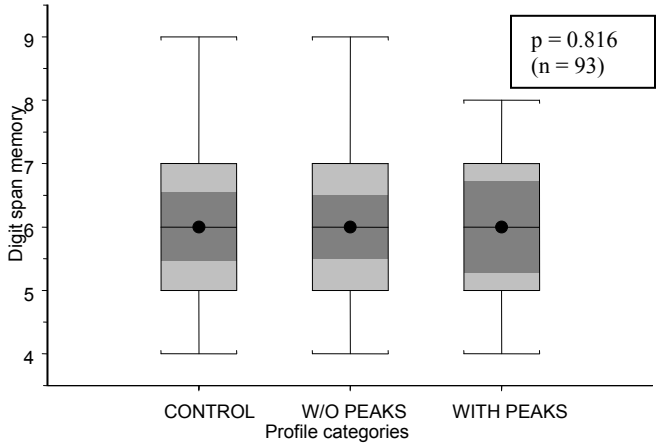

Figure 14: Comparison of the results obtained for the "Digit span memory" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

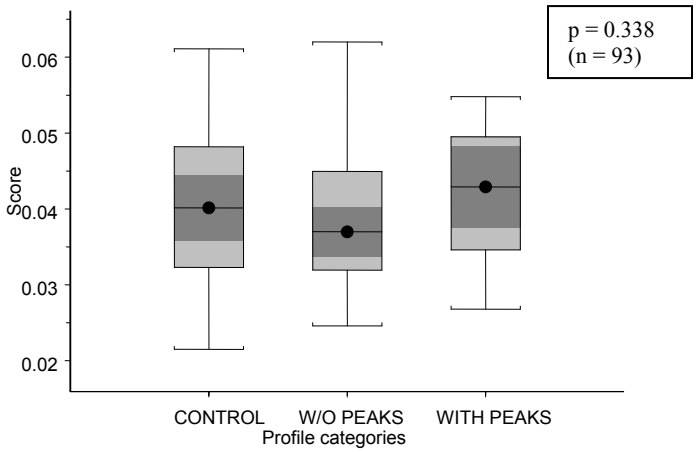

Figure 15: Comparison of the results obtained for the "Continuous tracking" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

C. Sensory tests

1. "Lanthony desaturated colour D-15"

The results of the comparison of the colour confusion index for the three exposure groups according to the profile category for the right eye and left eye are summarized in Table 11 and presented in Figure 16 for the right eye, and in Figure 17 for the left eye.

Table 11: Summary of the results obtained for the colour confusion index for the "Lanthony desaturated colour D-15" test in the three exposure groups according to profile category

	Exposure groups			p (Kruskal Wallis)
	Control	Exposed		
		Without peaks	With peaks	
CCI Right eye Median	1.125	1.243	1.199	p = 0.382 (n = 94)
CCI Left eye Median	1.139	1.118	1.062	p = 0.286 (n = 95)

Note: CCI = Colour confusion index

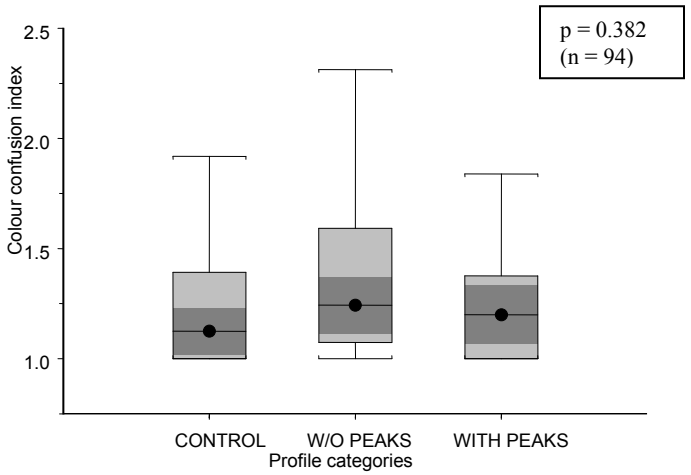

Figure 16: Comparison of the results obtained for the colour confusion index for the right eye for the "Lanthony desaturated colour D-15" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

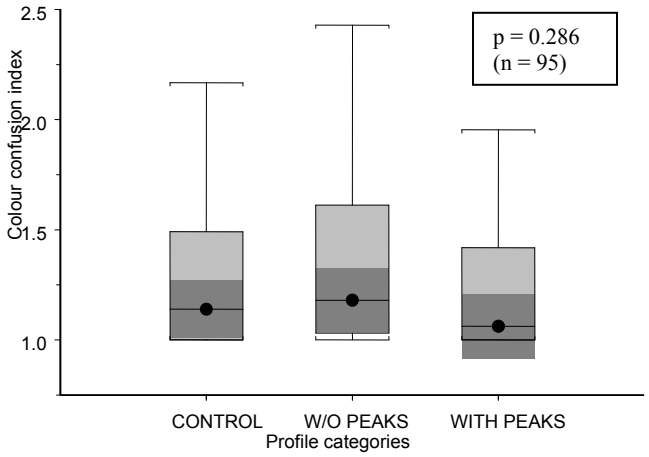

Figure 17: Comparison of the results obtained for the colour confusion index for the left eye for the "Lanthony desaturated colour D-15" test for the three exposure groups according to profile category. Note: *idem* Figure 2.

Based on these results, concentration peak exposure, as defined in this study, has no effect on the colour confusion index.

2. "Vistech VCTS 6000"

The results of comparison of the vision contrast threshold for the three exposure groups according to profile category for the right eye and left eye are presented in Table 12. No difference was identified between the groups.

Table 12: Summary of the results obtained for contrast vision for the "Vistech VCTS 6000" test for the three exposure groups according to profile category

Parameter measured	Frequencies (Cycles/degree)	Exposure groups			p (Kruskal Wallis)
		Control	Exposed		
			Without peaks	With peaks	
Vision contrast threshold Median right eye	**1.5**	35	35	35	0.979 (n = 93)
	3	85	85	85	0.480 (n = 93)
	6	70	70	70	0.121 (n = 93)
	12	55	32	32	0.831 (n = 93)
	18	15	15	12.5	0.496 (n = 93)
Vision contrast threshold Median left eye	**1.5**	35	35	35	0.554 (n = 94)
	3	85	85	85	0.862 (n = 94)
	6	70	70	70	0.404 (n = 94)
	12	32	32	32	0.705 (n = 94)
	18	10	12.5	10	0.683 (n = 94)

As with the neuropsychological tests, the results of this study show the lack of significant association between the exposure categorized according to the profiles and a change in visual function evaluated with the "Lanthony desaturated colour D-15" and "Vistech VCTS 6000" tests.

5.3.3.2 Exposure to peaks by considering the TWA concentration: stratification and one-factor analysis

While the data from this study revealed only some upper respiratory tract irritation that can be attributable to styrene, it seemed important to verify whether certain effects would be more associated with the concentration peaks rather than with the average exposure. This analysis was done by categorizing the exposure according to the three following groups: "Control group," "High group without peaks" (> 213 mg/m³ or 50 ppm without peaks), and "High group with peaks" (> 213 mg/m³ or 50 ppm with peaks). The statistical analysis performed with the Kruskal Wallis test demonstrated that the groups differed for only one aspect of mood (Arousal +) and one symptom (nasal irritation). The multiple comparison procedure showed that for arousal and for nasal irritation, the exposed groups were significantly different from the Control group ($p < 0.05$). Based on these data, exposure to peaks cannot be implicated in the reported effects (results not illustrated).

5.3.3.3 Exposure to peaks by considering the TWA concentration: two-factor analysis

In order to differentiate, in exposed subjects, the effects related to concentration peaks from those related to TWA concentrations, a two-way ANOVA was performed where it could be applied. Apart from the results obtained for the "Continuous tracking" test, based on the results summarized in Table 13, styrene exposure, whether expressed as an average obtained by dosimetry or by profile category, shows no statistically significant association with the results obtained for the neuropsychological tests ($p > 0.05$). For the "Continuous tracking" test, only the average exposure evaluated by dosimetry was significantly associated with the results obtained ($p = 0.005$). However, as with the analyses performed with the two exposure indicators considered separately (TWA concentration or profile category), gender was identified as being a modifying factor. After this latter factor was controlled, no association emerged between the average styrene exposure (evaluated by dosimetry) and the results for the "Continuous tracking" test.

Table 13: Results of the two-way ANOVAs of the neuropsychological tests

Neuropsychological test	Variables	p (2-way ANOVA) n = 60
Simple reaction time	Dosimetry	0.820
	Profile category	0.556
Colour-word stress	Dosimetry	0.700
	Profile category	0.596
Symbol digit matching	Dosimetry	0.491
	Profile category	0.114
Continuous tracking	Dosimetry	0.005
	Profile category	0.089

5.3.4 Results of simulation by modeling

A. Styrene concentration in the brain

Simulation of the brain styrene concentration was used to evaluate the impact of styrene inhalation exposure in terms of average and peak concentration on the styrene level in the brain. The PBTK model developed revealed that exposure by inhalation to concentration peaks led to a significant increase in the styrene level in the brain. Figure 18 and Figure 19 present at the same time the evolution in atmospheric exposure concentrations and the predicted concentrations in the brains of two workers, with the first categorized as “Without peaks” (Figure 18) and the second “With peaks” (Figure 19). On these figures, in Part A, the solid black line represents the point atmospheric concentrations during the work shift. The average of these point concentrations in the atmosphere for the described interval is represented by the blue line. In Part B, the black line represents the predicted brain concentrations corresponding to the point exposure concentrations described in the respective Part A. The blue line represents the result of modeling the average atmospheric exposure value for the corresponding described interval.

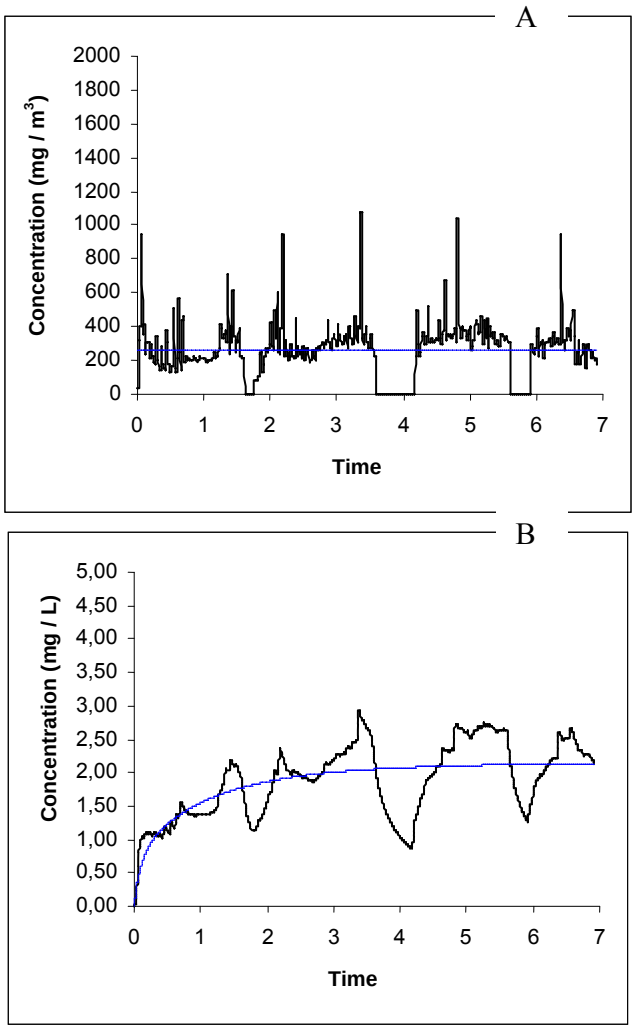

Figure 18: Results of the modeling of the predicted styrene concentrations in the brain from concentrations in the inhaled air for a worker categorized as "Without peaks." Note: In Part A, the black line represents the profile of the point concentrations in the inhaled air measured by PGC, and the blue line, the value of the average exposure during the described time interval. In Part B, the black line represents the concentrations predicted by the model in the brain following exposure to the profile in Part A, and the blue line, the modeled concentrations following exposure to the average of the profile in the described time interval

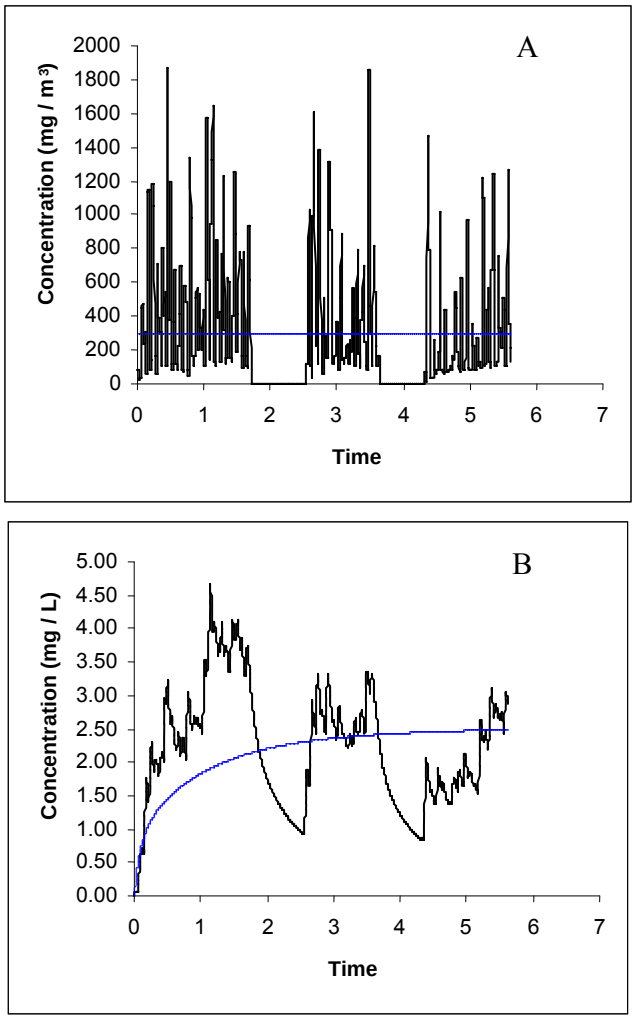

Figure 19: Results of the modeling of the predicted styrene concentrations in the brain (A) from the concentrations in the inhaled air for a worker categorized as "Without peaks." Note: *idem* Figure 18

From observation of these figures, one notes first that the modeled styrene concentration in the brain follows the evolution in the measured concentration in the inhaled air. In other words, with the two workers, an increase in the concentration in the inhaled air induces the same event in the brain, with a brief latency period. In addition, Figure 19 shows that the intensity of the increase in concentration in the brain seems higher for the worker with a "With peaks" profile than for the worker with a "Without peaks" profile (Figure 18).

B. Relationship between environmental exposure to styrene and urinary mandelic acid

In the 40 workers for whom urine samples were available, a high correlation was obtained between the styrene concentrations in the inhaled air and the urinary mandelic acid concentrations measured at the end of the work shift (R2 = 0.875; Figure 21).

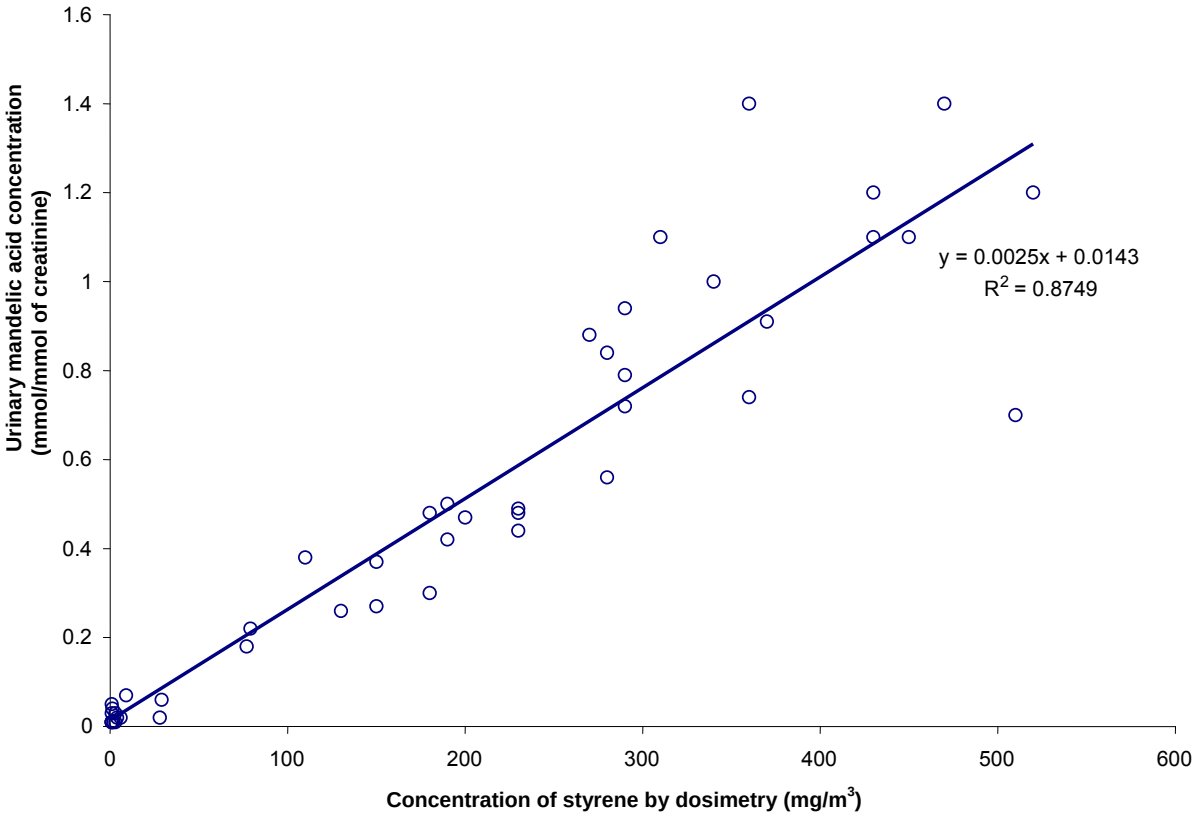

Figure 20: Relationship between the concentration of inhaled styrene and the urinary mandelic acid concentration

C. Modeling of the excretion of urinary mandelic acid following exposure to styrene alone

The excretion of urinary mandelic acid measured in workers is well described by the PBTK model developed. The coefficient of determination obtained is in fact high (R2 = 0.809) (Figure 20).

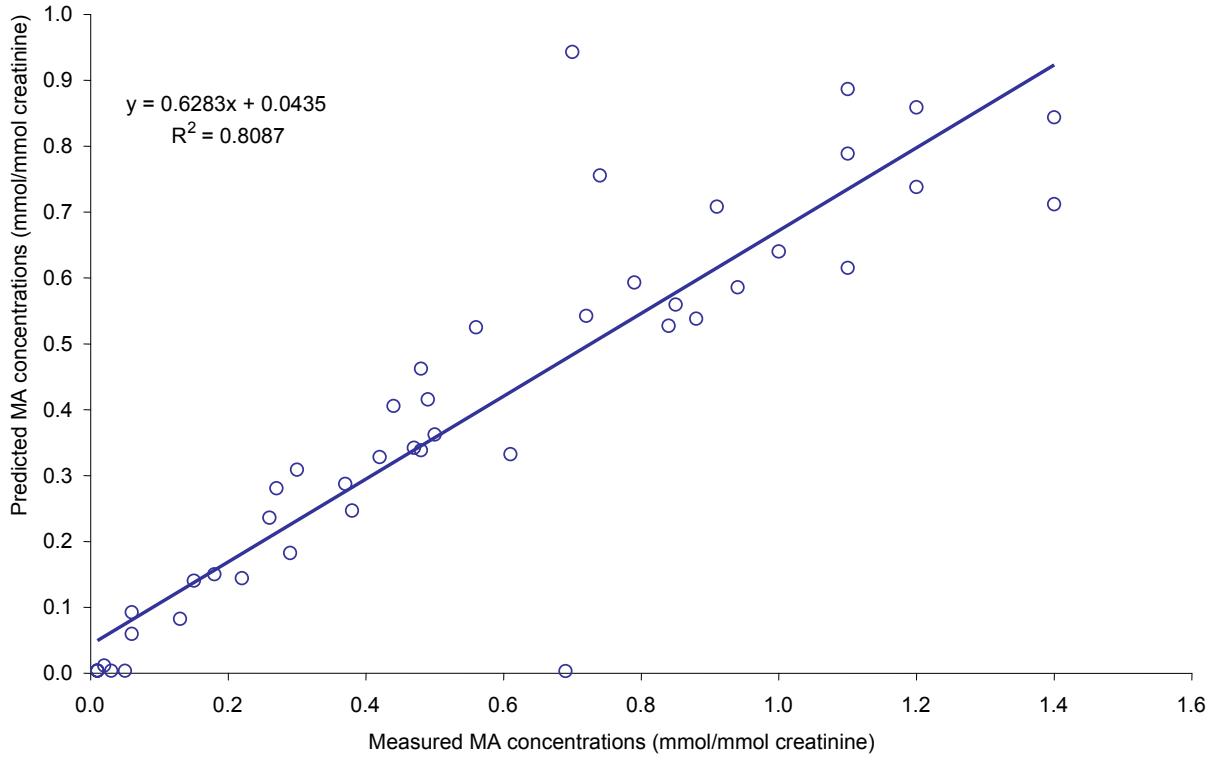

Figure 21: Comparison of the excretion of mandelic acid (MA) predicted by the developed PBTK model to the measured MA

D. Modeling of urinary mandelic acid excretion following simultaneous exposure to styrene and acetone

In the simulation of urinary excretion of mandelic acid in the context of simultaneous exposure to styrene and acetone, three scenarios were considered: with competitive or non-competitive interaction, and the absence of interaction (Figure 22). The results of the simulations show the absence of effect of simultaneous exposure to styrene and acetone on the predicted levels of mandelic acid. This lack of effect is in complete agreement with the existence of a very good correlation, mentioned above, between the mandelic acid levels and the styrene exposure observed in the workers (and confirmed by modeling), which would likely not have been the case if the presence of acetone, at the measured levels, had significantly changed the metabolism of styrene.

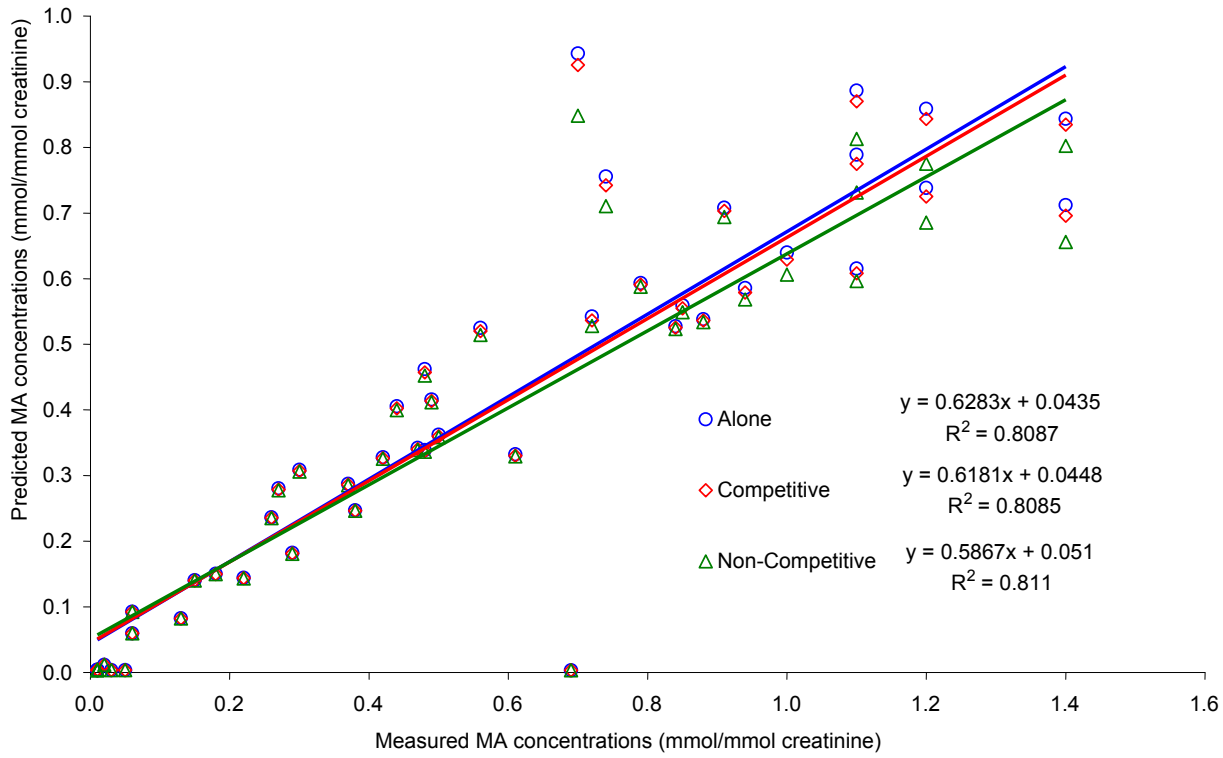

Figure 22: Relationship between the measured urinary MA concentration and that obtained by modeling by assuming a) the absence of metabolic interaction with acetone, b) a competitive metabolic interaction, and c) a non-competitive metabolic interaction

5.3.5 Results of the analysis of the exposure history

Since the exposure history could not be determined satisfactorily in the years preceding sampling (see section 5.3.1), the relationship between the cumulative styrene exposure duration and the neurotoxic effects studied could not be determined with the necessary scientific rigour. In addition, the exposure durations estimated in this study are shorter than those reported in other studies. In general, to verify the effect of exposure duration on styrene neurotoxicity, various authors consider a duration longer than eight or ten years (Viaene et al. 2001; Benignus et al. 2005; Triebig et al. 2009). The average for this variable in this study was 5.6 years and would be attributable, in this type of industry in Québec, to a particularly high turnover of personnel.

No information in the second part of the self-administered questionnaires led to the conclusion that the exposure levels reported by the workers and estimated by the experts may have been different in the years preceding this study. This evaluation is partially corroborated by the quantitative results presented (measured by occupational hygiene technicians in the context of

the health programs) in Table 14. The only historical exposure values available are those for laminators.

Table 14: Comparison of the arithmetic mean of the exposure levels of laminators

Establishment	Year(s) with historical values	Number of measurements		Historical exposure value	Exposure value in this study
		H	S	mg/m³	mg/m³
Plant F	2000	19	6	235	128
Plant H	2001	14	47	341	352
Plant I	2003	30	30	299	334
Plant K	2002	11	12	201	188
Plant M	1996-1998	10	18	100	201

Note: H = historical and S = study

In our opinion, since the nature of the manufactured products and processes in plant F changed between the measurements taken in 2000 and those taken during this study, this explains the observed difference; in 2000 the parts contained more resin and automation was not as extensive. Production in plants H, I and K remained unchanged and the results, for all practical purposes, are identical. We have no information that allows us to explain the difference for plant M.

Since the industrial processes in this industry generally did not undergo significant modifications during the observation period, we are led to conclude that the exposure levels generally remained homogeneous for all of the exposed workers during the 2000s.

The same conclusion can be drawn from an analysis of the Solvex database (35) available on the Web site of the Institut national de recherche et de sécurité INRS. It shows that the exposure of "Laminators-mould makers" in industry "2229A – Fabrication de pièces techniques à base de matières plastique" (manufacture of plastic-based technical components) was 96 mg/m³ for the 2001–2004 period, while it was 127 mg/m³ for the 2005–2008 period.

6. DISCUSSION

The FRPI industry in Québec, essentially consisting of small and medium-sized enterprises (average of 40 workers per establishment) has close to 6000 workers. Such a number should have made it easy to recruit a hundred or so volunteers. Numerous factors combined, thus making this task extremely difficult. In addition to this industry's historical reluctance, fearful that the results would be used for purposes other than scientific ones, was the RICQ board of directors' incorrect interpretation of the research protocol. It mistakenly believed that the study required worker exposure to concentrations exceeding the PEVs. In this very competitive Québec environment, each company tries to minimize leaked commercial information. As a result, respect for the confidentiality of information is a determining factor in establishing a relationship of trust with company directors. An underestimation by the researchers of the importance of this aspect and the very nature of the regulatory changes that occurred, during the project, to the system of "diseases" reportable to the public health director severely affected the conduct of this study. Numerous factors combined and affected how this study was carried out. These included the employers' fear that confidential information on the manufacturing process might be leaked or even their concern that high levels of metabolite would be reported to the public health director pursuant to the Regulation. These factors prevented the work being carried out according to schedule and were the reason for the delay in the project and the loss of establishments potentially interested in participating in this study.

In this study, we set up three groups of workers belonging to the same number of exposure categories. The "controls" were exposed to very low styrene concentrations (see justification below in the discussion). Exposure in the two other groups was categorized according to two levels, namely average and high. Each of these two groups was then divided into two sub-groups presenting an exposure "with" or "without" peaks. It is interesting to note that these groups were almost equal in size.

As previously indicated, the acetone used in the FRPI could *a priori* hinder the interpretation of the results. Acetone represents at most 36% of its limit value, while the value reached by styrene is 244% (Table 2). This analysis suggests that the potential contribution of acetone to any potential neurotoxic effect can reasonably be considered negligible. From a toxicokinetic standpoint, PBTK modeling of the impact of acetone on the biotransformation of styrene into mandelic acid is also minimal according to the results presented in Figure 22. The exposure-effects relationship could therefore be analyzed statistically by considering only styrene exposure.

In the present study, the effects of styrene exposure on the nervous system in workers in the FRPI were evaluated by means of a battery of tests used for evaluating the subjects' neuropsychological and neurosensory response. Three categories of tests were used: subjective evaluation questionnaires, neuropsychological tests, and sensory tests. The primary objective of this study was to verify whether the neurotoxic effects associated with styrene could be associated with concentration peaks, even at exposure levels not exceeding the TWAEV of 213 mg/m³.

In this study, the statistical approach consisted of comparing the distribution of the response to the various tests (dependent variable) among the styrene exposure groups (the independent

variable). Three groups of dependent variables were used: *i)* the scores for the self-evaluation questionnaires; *ii)* the results for the neuropsychological tests, and *iii)* the scores for the sensory tests. Styrene's impact on these results was studied by considering the exposure according to three scenarios: *i)* the time-weighted average concentration; *ii)* the profile category ("With peaks" and "Without peaks"), as well as *iii)* the combination of these two factors simultaneously.

First, the analysis addressed the relationship between the average styrene concentration calculated for one workday (approximately eight hours) and the three categories of tests. The presented results show that, apart from certain symptoms evaluated with subjective questionnaires, it was the absence of a link between styrene and the neurotoxic effects that was observed. In fact, for all of the neuropsychological and sensory tests, the scores obtained with the two exposed groups (average ± standard deviation: Average group, 137 ± 52 mg/m³; High group, 333 ± 95 mg/m³) did not differ statistically from those obtained with the controls (average ± standard deviation: 7 ± 8 mg/m³).

Regarding irritation with discharge from both the eyes and nose as well as throat irritation, the median obtained for the High group was significantly different from that for the Average group and Control group. Based on these data, a threshold for upper respiratory tract irritation can be established at an average concentration in the order of 137 mg/m³. This threshold was also described for two other symptoms, impressions of chest pressure and unpleasant taste.

Critical analysis of the studies that addressed styrene's toxic effects in exposed workers showed that eye and upper respiratory tract irritations were among the effects most frequently reported (Vyskocil et al. 1997). The present study confirms this result. In terms of the concentration threshold at which these effects can be observed, certain differences were noted. While Murata et al. (1991) more frequently reported irritation symptoms in workers exposed on average to 94 mg/m³, Harkonen (1977) as well as Viaene et al. (2001) reported them for exposure levels in the order of 172 and 150 mg/m³ respectively. The threshold of 137 mg/m³ established in this study therefore corroborates the one obtained by Viaene et al. (2001). The subjective nature of these symptoms may explain in whole or in part the differences with the other studies. However, it is important to note that the size of the sample in the study by Murata et al. (1991) was particularly small (11 men). Since the number of participants in the studies by Harkonen (1977) or by Viaene et al. (2001) was of the same order as the present study, the power given to these last results must be greater.

In the second part of the statistical analysis, by considering the profile category as the styrene exposure indicator, results similar to those obtained in the first part (TWA concentration as exposure indicator) were obtained. For four symptoms (fatigue, nasal discharge, the impression of unpleasant odours and unpleasant taste), a statistically significant difference was obtained in the Exposed group ("With peaks" and "Without peaks") compared to the Control group. For one symptom, eye discharge, only the Group "Without peaks" showed a median significantly different from that of the Control group. For all the other symptoms, as with the neuropsychological or neurosensory tests, no difference between the groups was noted. Based on these results, it is impossible to implicate or eliminate exposure to peaks as a possible explanatory variable for the reported effects.

To verify whether certain effects would be associated more with concentration peaks than with the average exposure, the statistical analysis was done by categorizing the exposure according to three groups: "Control group," "High group without peaks" (> 213 mg/m³ without peaks) and "High group with peaks" (> 213 mg/m³ with peaks). Comparison of the two exposed groups with the Control group showed that except for the "Arousal+" aspect of mood and nasal irritation, no difference between the groups was noted and that the results were similar, regardless of the exposed group considered. Based on these data, exposure to concentration peaks cannot be implicated in the reported effects.

The second specific objective of this study was to estimate workers' styrene exposure that could be associated with neurotoxic effects, from the measurement of urinary mandelic acid, and to predict, using a toxicokinetic model, the styrene concentrations in the brain that will be produced in relation to the exposure profiles recorded in the industries. Given the lack of neurotoxic or neurosensory effects, this objective could not be achieved in the framework of this project. However, it is interesting to note that in this study, by considering the sub-group of workers for which there were urine samples, a high correlation was obtained between the styrene concentrations in the inhaled air and the urinary mandelic acid concentrations measured at the end of the work shift (R2 = 0.875; Figure 20). These results corroborate those reported by Truchon et al. (1992). The relationship obtained by these authors (y = 0.0027x + 0.0079; R2 = 0.884) is in fact very close to the one observed in this study.

The lack of power could be mentioned as a possible explanation for the lack of neurotoxic or neurosensory effects. In fact, interindividual variability is large and the amplitude of the investigated effect remains low. However, analysis of the power showed that it was sufficient for the neurotoxic tests, for this type of analysis, at the effect amplitudes investigated. This analysis would consequently favour a lack of association between styrene exposure, at the levels described, and the neurological or sensory effects studied.

Other studies did not observe styrene-related neurotoxic effects, and the study of Triebig et al. (1989) whose reliability had been considered as high is a good example of this. While the average styrene concentration reached 1069 mg/m³ and that of the peaks 2556 mg/m³, for a battery of 12 tests, the authors did not note significant differences between the scores obtained for the exposed subjects and those observed for the controls (Vyskocil et al. 1997). In a study recently published by Seeber et al. (2009), even though the acute exposure reached 170 mg/m³ and the average chronic exposure for the duration of the work shift was 115 mg/m³ for 15 years (and higher average exposures of 213-426 mg/m³ in the past), no neurosensory effect was noted either with the "Lanthony desaturated colour D-15" test or the "Vistech VCTS 6000" test. Nevertheless, the statistical power of studies using neuropsychological and neurosensory tests, as does this study, could be improved. In fact, in order to offset interindividual variability, the use of a before-after type procedure would be appropriate. Each worker would therefore be his own control. In the context of this study, this approach would have required greater availability of the workers, which represented a possible additional restriction to their participation.

In the studies using neurobehavioural tests like those used in this study, the selection of the Control group is particularly critical. In fact, the sociodemographic and economic characteristics of these groups must be as close as possible to those of the exposed groups. We therefore preferred to use a Control group very weakly exposed (average of 7 mg/m³) and chosen in the

same company as the exposed group in order to limit the introduction of other biases that we considered more important. Note that in this, our analysis differs partially from that of Toppila (2006) who considered that this approach might lead to an underestimation of the risk.

In summary, under the exposure conditions observed here, the results of this study do not support an association between styrene exposure and the alterations in the central nervous system as evaluated with the neuropsychological tests ("Simple reaction time"; "Colour-word stress"; "Symbol digit matching"; "Digit span memory"; "Continuous tracking") and neurosensory tests ("Lanthony desaturated colour D-15"; "Vistech VCTS 6000") that were used. This lack of association was observed with the grouping criteria considered, namely the TWA concentration, the profile category in the entire Exposed group, and the combination of the two factors simultaneously.

These results are unexpected, since several studies have shown that styrene exposure affects the nervous system, in particular the colour confusion index (Bowman 1982). Since the power given to our results was high, on the basis of these data one can reasonably consider that, at the exposure concentrations described and for the duration of exposure of the workers in our study, styrene has no appreciable effect in the battery of neurosensory and neuropsychological tests used. Nevertheless, in several studies, it has been demonstrated that styrene exposure duration (more than eight years) may have an effect on the nervous system (Viaene et al. 2001; Benignus et al. 2005; Triebig et al. 2009). It is an important parameter to be considered in studying the chronic effects associated with styrene. Unfortunately, in this study, this association could not be analyzed. As previously mentioned, the information necessary to properly examine the historical data was insufficient. In fact, since the evolution in the production levels, like that in the means of exposure control, was not well documented in all of these establishments, this analysis could not be done satisfactorily. In addition, because the average exposure time for the workers in our study was only 5.6 years, it could explain in whole or in part the lack of association between styrene exposure, at the levels described, and the nervous system effects as evaluated in this study. Nonetheless, the results of this study support the regular occurrence of concentration peaks in the FRPI. These concentrations remain high, and occupational hygienists must continue to pay particular attention to them.

Even though the battery of tests used consisted only of standardized and validated tests for evaluating the neurological functions that could be disrupted by styrene exposure, the hypothesis that results different from those obtained in this study could have been observed with another battery of tests cannot be ruled out. In particular, tests that can induce additional fatigue or that require more sustained attention and concentration may possibly have revealed a neurotoxic effect associated with styrene exposure. To our knowledge, such tests have not yet been used to evaluate styrene's neurotoxicity in workers.

7. CONCLUSIONS AND RECOMMENDATIONS

The question of revising the eight-hour time-weighted average exposure value (TWAEV) for subjects that are occupationally exposed to styrene is now receiving particular attention from regulatory organizations. Currently, the values for the TWAEV and STEV in Québec are 213 mg/m^3 (50 ppm) and 426 mg/m^3 (100 ppm), respectively.

Studies published in the scientific literature and carried out on workers exposed to styrene vapours in the workplace suggest that neurotoxic effects are probably the most sensitive indicators of styrene toxicity. However, the data available do not allow a NOAEL and/or a LOAEL to be established for neurotoxic effects following acute or chronic inhalation exposure. Only a few studies discuss the distinction between the short-term effects and the long-term effects.

A few studies, including one by our group, have been carried out on styrene-concentration time profiles in the fibreglass reinforced plastics manufacturing industry. They have demonstrated that concentration peaks reaching several times the time-weighted average concentration occur frequently in workplaces. These must be taken into consideration in evaluating acute and chronic health effects following styrene exposure in the workplace.

Several studies suggest the presence of prenarcotic symptoms, a lengthening of the reaction time, and a loss of colour perception for styrene concentrations exceeding 213 mg/m^3 for 15 minutes. Low scores in other neurobehavioural tests may occur at peak concentrations above 300 mg/m^3.

The aim of this project was to verify the impact of styrene concentration peaks on this solvent's toxicity with exposed workers. In the literature, we found several definitions of what should be considered a "peak." We defined an exposure with peaks in terms of the difference in relation to the average time-weighted concentration and the total duration of this difference.

We carried out the project in two phases. The objective of Phase I was to verify whether acute, early, and reversible neurotoxic effects occurred in volunteers exposed under controlled conditions. In this phase, we evaluated the effects with a battery of neurotoxicity tests widely used globally. The volunteers were subjected to five styrene exposure scenarios for six hours: three stable concentrations, and two variable concentrations with peaks not exceeding the STEV. The results show that a six-hour exposure to concentrations not exceeding the TWAEV and the STEV has no effect on neurosensory performances, on cognitive capacities, on variations in mood, or on the appearance of symptoms. Nevertheless, the design of such an experimental study is necessarily different from the actual exposure conditions of workers in their work environment. Hence, our subjects had never been exposed to styrene before this study, the exposure duration was only six hours, and the subjects were resting during the exposure (low pulmonary ventilation). Consequently, we could only evaluate the acute component of the potential effects for resting subjects.

The main objective of the second phase was to verify whether styrene's neurotoxic effects are related to styrene concentration peaks for workers exposed in a plant, even to average concentrations not exceeding the TWAEV of 213 mg/m^3.

We observed significant styrene exposure peaks in the fibreglass reinforced plastics industry. In some cases, the measured styrene values even exceeded the standards prescribed by the *Regulation respecting occupational health and safety*. We used the same battery of early screening tests for neurotoxicity as in Phase I.

Our results, obtained in a cohort of approximately one hundred workers, namely a larger sample than in most of the studies, indicate that at the exposure concentrations described and for the duration of exposure of the workers in our study, styrene has no appreciable effect on the battery of neurosensory and neuropsychological tests used.

In summary, several factors could have influenced the results of this study: the definition of peaks, the statistical power that could be improved, or the duration of the average exposure of the workers (in our study, it was only 5.6 years). This duration may not be sufficient for evaluating chronic effects. Furthermore, our study does not allow short-term effects to be differentiated from long-term effects. The use of a study design by which the subjects would undergo the same battery of tests before the start and at the end of a day of exposure would perhaps provide information on the acute effects.

The results of our study do not suggest that the Québec styrene exposure standards for workers exposed under conditions similar to those in our cohort must be lowered. In particular, our results do not confirm any toxic effect on the nervous system associated with exposure to concentration peaks as we have defined them. We recognize that our results contradict those obtained by some researchers, but are consistent with those that have been published by others. In addition, this conclusion suggests that the battery of validated tests that we used is the one that allows styrene's most sensitive toxic effects to be measured and that offers the best guarantee of prevention of harmful health effects on the exposed individuals.

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9. APPENDICES

APPENDIX I: RECRUITMENT OF PARTICIPATING ESTABLISHMENTS

Step 1: Creation of a list of Québec establishments with workers potentially exposed to styrene

Styrene is used in economic activity sectors other than the manufacture of reinforced plastic products. However, a preliminary evaluation of the industrial processes revealed that processes involving a polyester resin in styrene were the best adapted to the needs of this project: direct contact of workers with styrene, a large pool of exposed workers, production tasks involving very little change of location during the measuring day, and manual tasks favourable to producing exposure peaks. We therefore focused our search for establishments on this activity sector by using two databases that can be consulted on-line, that of the NPRI and that of ICRIQ.

The establishments retained from this list were chosen on the basis of either of the two following criteria:

1. The establishment submitted, for the year 2002, a report about styrene to Environment Canada's National Pollutant Release Inventory (NPRI) and was classified with one of the following NAICS economic activity codes:

326191	Plastic Plumbing Fixture Manufacturing
326193	Motor Vehicle Plastic Parts Manufacturing
326198	All Other Plastic Product Manufacturing
336211	Motor Vehicle Body and Trailer Manufacturing
336360	Motor Vehicle Seating and Interior Trim Manufacturing
336611	Ship Building and Repairing
336612	Boat Building

2. The establishment was simultaneously on both lists extracted from the ICRIQ according to NAICS codes 326191, 326193, 326198, 336211, 336360, 336611, 336612 or according to a list[1] of product codes.

While the establishments from the NPRI list are generally few in number, this source of information readily identifies the major users of styrene in Québec.

Step 2: Creation of a list of establishments suitable for recruitment

The establishments were chosen according to the following criteria:

- Amounts of styrene used,
- Manufacture of fibreglass objects,
- Number of potentially exposed workers
- Demonstration of some interest during the first telephone conversation, or
- Participation in Phase I of the study.

[1] The list of manufactured products used to identify the establishments is presented at the end of this appendix.

This sub-set of establishments should represent the one with the best cost-benefit ratio of the energy to be deployed to obtain their participation.

Search for establishments according to the ICRIQ codes for manufactured products

PLASTICS - MANUFACTURING	
PIPES AND PIPE FITTINGS, PLASTIC	Pipe fittings, reinforced plastic
	Industrial pipe of reinforced plastic
PLASTIC CONTAINERS	Feeding troughs, fish hatchery, reinforced plastic
	Basins, fish hatchery, reinforced plastic
	Tool boxes, pickup truck/van, reinforced plastic
	Containers for trash, reinforced plastic
	Containers for abrasives, reinforced plastic
	Containers for recyclable materials, reinforced plastic
	Storage tanks, fuel oil, reinforced plastic (commercial or industrial)
	Storage tanks, fuel oil, reinforced plastic (residential)
	Storage tanks, drinking water, reinforced plastic
	Storage tanks, gasoline, service station, reinforced plastic
	Tanks, reinforced plastic
	Tanks, industrial process, reinforced plastic
	Tanks, pressure, industrial process, reinforced plastic
	Silos, reinforced plastic
PLASTIC DOORS AND WINDOWS	Windows, reinforced plastic
	Doors, garage, reinforced plastic
	Doors, exterior, reinforced plastic
	Doors, patio, reinforced plastic
PLASTIC SANITARY FIXTURES	Bathtubs, whirlpool, acrylic
	Bathtubs, whirlpool, synthetic marble
	Bathtubs, whirlpool, reinforced plastic
	Bathtubs, synthetic marble
	Bathtubs, reinforced plastic
	Shower bases, acrylic
	Liners, bathtub, acrylic
	Laundry tubs, acrylic
	Showers, acrylic
	Showers, synthetic marble
	Showers, reinforced plastic
	Sinks, acrylic
	Wash basins, acrylic
	Wash basins, synthetic marble
	Panels, wall, bathtub, acrylic
	Panels, wall, bathtub, reinforced plastic
	Toilets, synthetic marble
PLASTIC BUILDING PRODUCTS	Awnings, reinforced plastic
	Balconies or patios, reinforced plastic
	Ponds, landscaping, reinforced plastic
	Prefabricated buildings, reinforced plastic
	Ducts, ventilation, reinforced plastic
	Tops, counter, synthetic marble
	Cisterns, reservoir, reinforced plastic
	Septic tanks, reinforced plastic
	Interceptors and separators, grease/oil/sediment, reinforced plastic
	Stair runners, reinforced plastic
	Building panels, reinforced plastic
	Flooring panels, non-slip and fireproofing
	Insulation panels, composite roofing
	Panels, reinforced plastic

	Shapes, pultruded reinforced plastic
	Sheds, reinforced plastic
	Rods, concrete, pultruded reinforced plastic
PLASTIC HOUSEHOLD PRODUCTS	Bathroom accessories, synthetic marble
	Planters, reinforced plastic
PLASTICS SUBCONTRACTING	Reinforced plastics: filament winding, subcontractor
	Reinforced plastics: cold press moulding, subcontractor
	Reinforced plastics: contact moulding, subcontractor
	Reinforced plastics: vacuum bag moulding, subcontractor
	Reinforced plastics: compression moulding, subcontractor
	Reinforced plastics: infusion moulding, subcontractor
	Reinforced plastics: injection moulding, subcontractor
	Reinforced plastics: projection moulding, subcontractor
	Reinforced plastics: pultrusion moulding, subcontractor
	Reinforced plastics: resin transfer moulding, subcontractor
	Reinforced plastics: inflatable bladder moulding, subcontractor
	Reinforced plastics: vacuum moulding, subcontractor
	Reinforced plastics high performance fiber: inflatable bladder moulding, subcontractor
	Reinforced plastics high performance fiber: cold press moulding, subcontractor
	Reinforced plastics high performance fiber: contact moulding, subcontractor
	Reinforced plastics high performance fiber: compression moulding, subcontractor
	Reinforced plastics high performance fiber: infusion moulding, subcontractor
	Reinforced plastics high performance fiber: injection moulding, subcontractor
	Reinforced plastics high performance fiber: pultrusion, subcontractor
	Plastic laminating, metal/wood products
OTHER PLASTIC PRODUCTS	Barn equipment and supplies, reinforced plastic
	Markers, boundary, reinforced plastic
	Casings, gear box, reinforced plastic
	Scaffolding, dielectric, reinforced plastic
	Ladders, insulated, reinforced plastic
	Ladders and step ladders, reinforced plastic
	Moulds, reinforced plastic
	Bucketwheel and ladle booms, reinforced plastic
	Panels, inground swimming pool, reinforced plastic
	Machined parts, electrical insulation, reinforced plastic
	Trays, serving, reinforced plastic
	Lamp posts for roadway lighting, playing fields, etc., reinforced plastic
	Floating docks, reinforced plastic
	Tank lining, reinforced plastic
	Capping boards, electrode, reinforced plastic/polymer concrete
	Composite cleats (wood/plastic)
TRANSPORT PRODUCTS – MANUFACTURING	
TRUCK BODIES AND TRAILERS	Bodies, light truck, reinforced plastic
	Bodies, van, reinforced plastic
	Trailers, snowmobile or all-terrain, reinforced plastic
	Truck bodies, reinforced plastic
	Sleighs, snowmobile, reinforced plastic
PLASTIC AUTOMOBILE PARTS	Spoilers, automobile, reinforced plastic
	Deflectors, truck, reinforced plastic
	Sun visors, exterior, truck or pickup truck, reinforced plastic
	Parts, bus, reinforced plastic
	Parts, truck, reinforced plastic
	Parts, automobile, reinforced plastic
	Luggage racks, exterior, bus, reinforced plastic

SHIPS AND SHIP PARTS	Boats, fishing, reinforced plastic
BOATS AND BOAT PARTS	Canoes, reinforced plastic
	Canoes, aramide fiber reinforced
	Boats, row, reinforced plastic
	Hulls, watercraft, reinforced plastic
	Motor boats, reinforced plastic
	Boat equipment, reinforced plastic
	Seats, boat, reinforced plastic
	Sailboats: catamarans
	Sailboats: drifters
	Sailboats: multihulls
	Sailboats: keelboats
	Yachts
	Yachts, motor-yachts and yacht parts (custom)
OTHER MANUFACTURED PRODUCTS	
LEISURE ARTICLES	Water slides, outdoor centre, reinforced plastic

APPENDIX II: MEDICAL QUESTIONNAIRE

QUESTIONNAIRE 1

Variables:

1. Family name, first name: _____

   Address: _____

   _____

   _____

   Telephone number: _____

2 Date of birth: _____

3. Weight: _____

   Height: _____

4. Last year of school completed: _____

5. Are you a smoker?

   no o

   yes o | ***If so***, how many per day? _____

6. How many glasses of alcoholic drinks (glass of beer or glass of wine or glass of spirits) do you drink every week?

   _____

7. Do you use a computer?

   _____

8. Do you play video games? How many hours per week?

_____

9. Do you take medication regularly?

no o yes o | ***If so***, what category of medications:
- o Drugs for the heart or blood pressure
- o Drugs to sleep, relax
- o Allergy medications
- o Other _____

State of health:

10. In the last 12 months, have you had one of the following diseases which was confirmed by a physician:

Disease	***Yes***	***No***
Blood pressure		
Heart (heart problem)		
Lung problem (emphysema, chronic bronchitis, asthma)		
Kidney problem (renal insufficiency)		
Diabetes		
Liver disease (hepatitis, cirrhosis)		
Digestive problem (ulcer, colitis, diverticulitis)		
Neurological problem (epilepsy, meningitis)		
Endocrine problem (hypo & hyperthyroidism)		
Myopia		
Daltonism		
Allergic problem		

11. In your opinion, is your state of health:

poor o average o good o excellent o

12. In the last 6 months, have you had one of the following symptoms at the end of your work shift:

Symptom	***Yes***	***No***
Excessive fatigue		
Concentration difficulty (difficulty paying attention to a task such as reading, driving, listening to television)		
Eye and nose irritation (red eyes, runny nose)		
Headache		
Dizziness		
Nausea		
Loss of appetite		
Palpitations		
Sleeping problems (difficulty falling asleep, frequent awakening, nightmares)		
Pins and needles sensation, numbness in legs and hands		
Irritability, impatience, abnormal nervousness		
Depressed mood, sad thoughts, lack of interest, social withdrawal, feeling like crying		
Other: _____		
Other: _____		
Other: _____		
Other: _____		

Thank you for your cooperation!

APPENDIX III: EXPOSURE QUESTIONNAIRE

Questionnaire 2

Q. 1 Identification

_____ _____
Family name First name

CURRENT EMPLOYER

Q. 2 Name of current employer: _____

Q. 3 Current job title: _____

Q. 4 Date when you began working at current job: _____

Q. 5 Describe your tasks in your current job:

_____

_____

_____

Q. 6 Do you carry out your task at a single workstation during the day or do you have to frequently change location in the plant?

ڤ A single workstation ڤ Frequent changes in location

Q. 7 Do you <u>usually</u> wear a respirator to do your work?

ڤ no ڤ yes

Q. 8 Did you previously perform other duties with your current employer?

ڤ no ڤ yes

Q. 9 If so, which ones? (Indicate the starting and ending dates – begin with the most recent)

_____ to _____

_____ to _____

_____ to _____

_____ to _____

PREVIOUS JOBS

Q. 10 Enter below all the previous jobs that you have held. Start with the most recent one.

Number	From	To	Job	Company
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Example:	1992	1995	Pump attendant	Petro-Canada in St-Lin

Q. 11 In the list below, indicate the products that were used in production or that were used in your previous jobs. Write the job number(s) from the previous question beside the corresponding product.

► Fibreglass resins ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Inks and dyes ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Glues or adhesive substances ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Gasoline or fuel ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Paint, colorant or varnish ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Solvent, stripper or degreaser ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Weld ف no ف yes Job number(s): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Other industrial chemical product ڤ no ڤ yes Job number(s): _____

(specify, e.g., lead, mercury): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

► Other industrial chemical product ڤ no ڤ yes Job number(s): _____

(specify, e.g., lead, mercury): _____

If so, describe the use of the product and the tasks performed:

_____

_____

_____

Q. 12 In your free time, outside of work, do you carry out a pastime that brings you in contact with the following products? ڤ no ڤ yes

If so, Check:

ڤ Fibreglass resins
ڤ Inks and dyes
ڤ Glues or adhesive substances
ڤ Gasoline or fuel
ڤ Paint, colorant or varnish
ڤ Solvent, stripper or degreaser
ڤ Weld
ڤ Other industrial chemical products (specify, _____)

Q. 13 Do you now have a second job that may expose you to the following products?

ڤ no ڤ yes

If so, Check:

ڤ Fibreglass resins
ڤ Inks and dyes
ڤ Glues or adhesive substances
ڤ Gasoline or fuel
ڤ Paint, colorant or varnish
ڤ Solvent, stripper or degreaser
ڤ Weld
ڤ Other industrial chemical products (specify, _____)

APPENDIX IV: CONSENT FORM

CONSENT FORM
INFORMATION FOR PARTICIPANTS
Effects of concentration peaks on styrene neurotoxicity in the fibreglass reinforced plastics industry

Researchers

Adolf Vyskocil, Ph.D., associate clinical professor, Department of Environmental and Occupational Health, Université de Montréal (SEST)
Claude Viau, D.Sc., full professor, SEST
Michel Gérin, Ph.D., full professor, SEST
Robert Tardif, Ph.D., associate professor, SEST
Bernardette Ska, Ph.D., full professor, School of Speech Pathology and Audiology, Université de Montréal
Alice Turcot, M.D., Direction régionale de la santé publique de Lévis
Ginette Truchon, Ph.D., researcher, Institut de recherche Robert-Sauvé en santé et en sécurité du travail
Daniel Drolet, M.Sc., chemist, Institut de recherche Robert-Sauvé en santé et en sécurité du travail

Description of the project

Styrene is a volatile solvent that is widely used in some industries. Its permissible average exposure value for 8 hours provided for in the Québec *Regulation respecting the quality of the work environment* is currently 213 mg/m^3. This value is also in force in other countries. But there is some question whether this value properly protects the health of people exposed to styrene 8 hours per day, 5 days per week throughout their entire working life. Some researchers propose reducing this value because studies indicate that there may be effects on the nervous system in workers in the fibreglass reinforced plastics industry. The problem is that these studies do not take into account the fact that the styrene concentration in the companies is not always the same. The smell is stronger at certain times of the day and these are called *exposure peaks*. The main objective of this study is therefore to verify whether the neurotoxic effects are linked to styrene exposure peaks. If, as we believe, peaks play an important role in the appearance of effects on the nervous system caused by styrene, the permissible average exposure value will have to be lowered to take into account the existence of peaks.

The effects that we will measure are preclinical and reversible effects. This means that these effects have no impact on a person's normal functioning in common situations of everyday life.

The aim of this study is to measure the neurotoxic effects on 120 workers, non-smokers if possible, in the fibreglass reinforced plastics industry who are exposed to variable concentrations of styrene.

Conducting the study

Before participating in this study, you must answer a short medical questionnaire in order to obtain the assurance that you are in good health.

The exclusion criteria are the following: people that regularly take medication (to control asthma or heart problems, analgesics, antibiotics, anticonvulsants) or consume three or more alcoholic beverages a day, people treated for psychiatric problems, workers with a chronic problem of the lungs, liver or kidneys, diabetic individuals, people with a history of alcoholism or drug dependency, individuals with documented occupational exposure to substances that are toxic to the nervous system (mercury, lead, solvents, etc.) which could interfere with the tests used in the study, people with a history of head trauma.

You will then be asked to remain at work for approximately one hour and fifteen minutes longer on a Tuesday and on the following Wednesday or Thursday. For one hour, you will undergo a series of tests that will indicate to us whether styrene has minor effects on your nervous system under normal working conditions. These tests are grouped into three categories. The first category consists of 2 tests to check your senses (colour vision, contrasts). The second category consists of 5 tests to check mainly your reaction time, your memory, your reasoning ability, and your manual dexterity. Finally, the third category consists of 2 tests, namely two questionnaires to evaluate your mood and your symptoms.

To verify the average styrene concentration in the air in your breathing zone, you will have to wear small passive dosimeters for the entire day on Tuesday, Wednesday and Thursday. For 4 to 8 hours, a technician will continuously measure the styrene concentrations by maintaining a sampling probe as close as possible to your breathing zone.

Confidentiality

All the collected information will be handled anonymously and will be used only for the purposes of this study. All data of a personal nature, including the results of urine analysis, will be kept in a locked file. However, possible situations of exceedence of styrene exposure standards will be handled as indicated in the "Release of results" paragraph.

The researchers conducting this study will not publish any information identifying you personally and will not make this information available to anyone outside the study. One exception to this rule involves the legal obligation of reporting mandelic acid results to the public health director, as indicated in the "Release of results" paragraph. Personal information will be destroyed two years after publication.

Freedom to participate and to withdraw

Your participation is voluntary. You can participate in our study even if you choose not to supply a urine sample, provided that you comply with the other conditions. You may decide to withdraw from the study at any time by communicating with the principal investigator without having to justify your decision.

Expected compensation

You will receive an amount of $100, in consideration of the additional time that you will have to spend in your workplace during the week. This total amount first includes a sum of $70 for wearing a dosimeter for two work shifts and being present for approximately one hour and fifteen minutes after 2 work shifts; you will receive half of this amount if you participate for only one day out of the two. You will be given an additional amount of $10 for wearing a dosimeter for the third work shift as well as $20 if you participate in all the steps.

Resource person

If you have any questions before or during the study, you may communicate at any time with the principal investigator: Adolf Vyskocil by telephone at (514) 343-6146 or by e-mail at adolf.vyskocil@umontreal.ca. For all ethics-related questions, you may contact Dr. Vincent Castellucci at (514) 343-6300.

Signatures

I have read and understood the content of this form. I certify that it has been explained to me verbally. I have had the opportunity to ask all my questions about this study and they were answered to my satisfaction.

I know that I am free to participate and that I remain free to withdraw from this study at any time by verbal notice.

I certify that I have been given sufficient time to come to my decision.

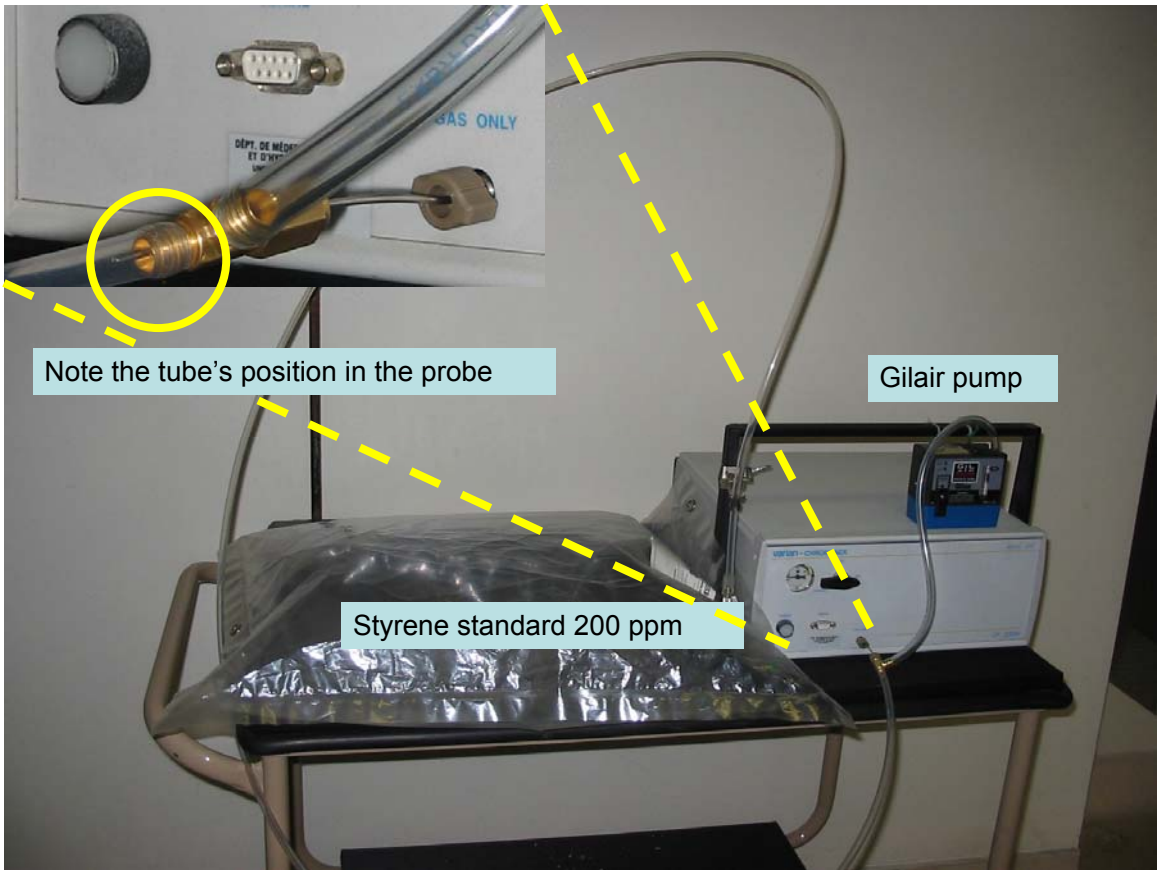
I, the undersigned, agree to participate in this study.

_____	_____	
Name of participant	Signature	Date
_____	_____	
Name of witness	Signature	Date

I certify that I *a)* have explained to the signatory the terms of this consent form; *b)* have answered the questions that he/she asked me about it; *c)* have clearly indicated to him/her that he/she is free to end his/her participation in the present research project; and *d)* will give him/her a signed copy of this form.

_____	_____	
Name of researcher	Signature	Date

APPENDIX V: APPARATUS FOR STYRENE EXPOSURE SAMPLING BY GAS CHROMATOGRAPHY



APPENDIX VI: PRESENTATION OF SYMPTOMS AND MOOD STATES DESCRIBED FROM THE SELF-EVALUATION QUESTIONNAIRE

Moods		Symptoms	
Attention +	**Attention -**	Headache	Eye discharge
Energetic	Passive	Dizziness	Blurred vision
Active	Vulnerable	Nausea	Nasal irritation
Concentrated	Ineffective	Fatigue	Nasal discharge
Stress +	**Stress -**	Chest pain or pressure	Impression of unpleasant odour
Calm	Stressed	Constant cough	Throat irritation
Rest	Stress	Shortness of breath	Impression of unpleasant taste
Relaxed	Tense	Eye irritation	Skin irritation
			Vertigo or loss of consciousness

APPENDIX VII: ADJUSTMENT OF PROTOCOL – PRELIMINARY STUDY

In the months of November and December 2003, we did the verification of the complete protocol in two small plants in Montréal on 6 workers sampled with a chromatograph. This allowed us to make adjustments to all the logistics.

Two new information documents (apart from the consent form) were prepared to explain the project clearly to the workers.

We also adjusted the protocol on two visits to solicit owners and workers:

a) During the first visit (professor + technician in charge of all the measurements): (1) the boss was informed about the project; (2) the plant was visited to identify locations for conducting the neurotoxicity tests; (3) the possible number of volunteers was discussed; and (4) the boss was given envelopes with all the forms for the volunteers. The boss distributed the envelopes to the workers who could communicate with the professors by telephone, if they had any questions.

b) During the second visit (professor + industrial hygienist), the volunteers' questions were answered, questionnaires were collected, and with each volunteer, his/her questions were clarified.

Toxicokinetic modeling was adapted to the needs of this study and the styrene concentrations in the brain were modeled for the 6 workers. The results of this preliminary study were presented to the X2004 conference in Utrecht.

APPENDIX VIII: PRESENTATION OF OBJECTIVES AND ASPECTS OF PHASE II OF THE PROJECT TO THE RICQ

Effects of concentration peaks on styrene neurotoxicity in the fibreglass reinforced plastics industry – Phase II

Department of Environmental and Occupational Health
Faculty of Medicine

Charles Beaudry, Occupational hygienist

Aspects of the presentation

- Objectives of this presentation
- Reminder about the research project
- Candidate selection criteria
- Recruitment of establishments
- CSST Schedule I committee
- SIRC research project
- Conclusions

Objective of this presentation

- To obtain the active participation of the RICQ in Phase II of the styrene study
  - Sharing of information to promote this participation
  - Comparing perceptions of the advantages and disadvantages of this participation

Reminder about the research project

Objectives of the project

- To verify whether neurotoxic effects are linked to styrene concentration peaks
- Recommendation about the use of the
  - PEV-TWAEV (8h) – 50 ppm (213 mg/m³)
  - PEV-STEV (15 minutes) – 100 ppm

  For subjects occupationally exposed to styrene

Reminder about the research project

Phase 1

- Activities in Phase I

- Evaluation of volunteers in the laboratory
- 0.25 and 50 ppm with and without peaks, 6 hours, once

- Conclusions of Phase I
  - No specific contribution of the concentration peaks to the neurotoxic effect
  - Exposure too short? Necessary cumulative effect of the exposure peaks?

Reminder about the research project

Phase 2

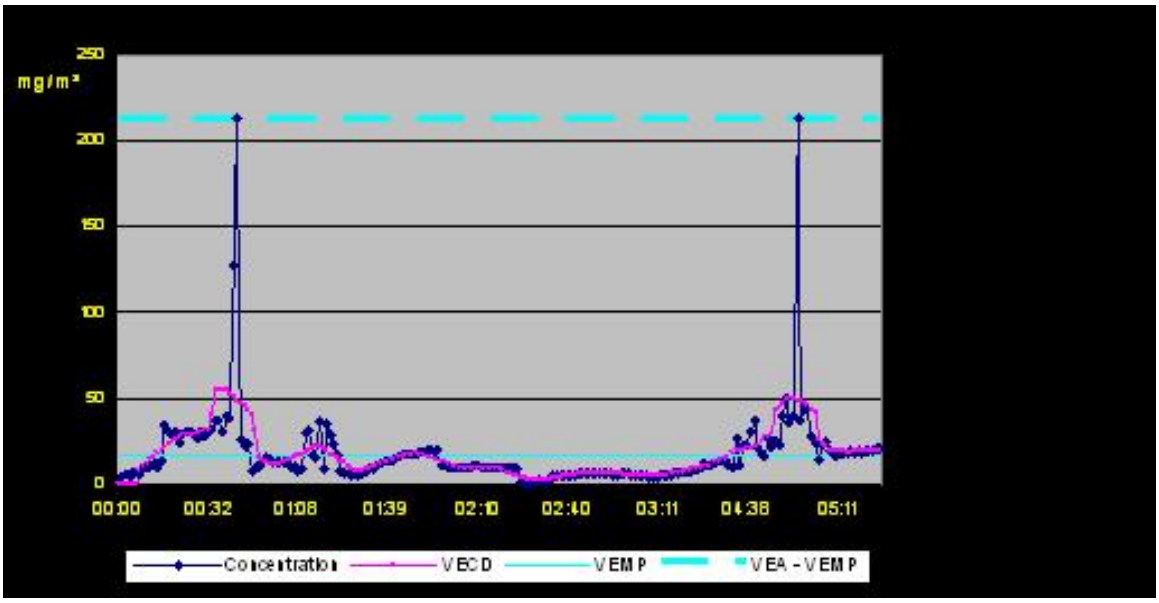
- Measurements in establishments

- Analysis of the exposure history

- Type of exposure sought
  - Unexposed worker - Control
  - Exposed worker - Without peaks
  - Exposed worker - With peaks

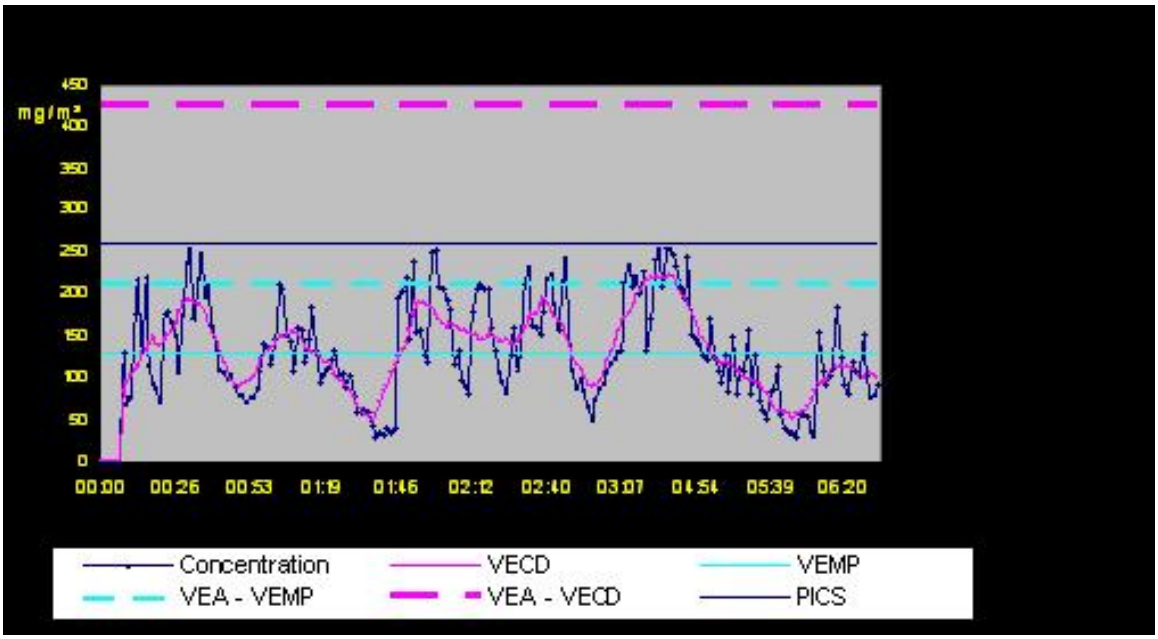
Candidate selection criteria

Criteria related to the exposure level (Unexposed worker – Control)



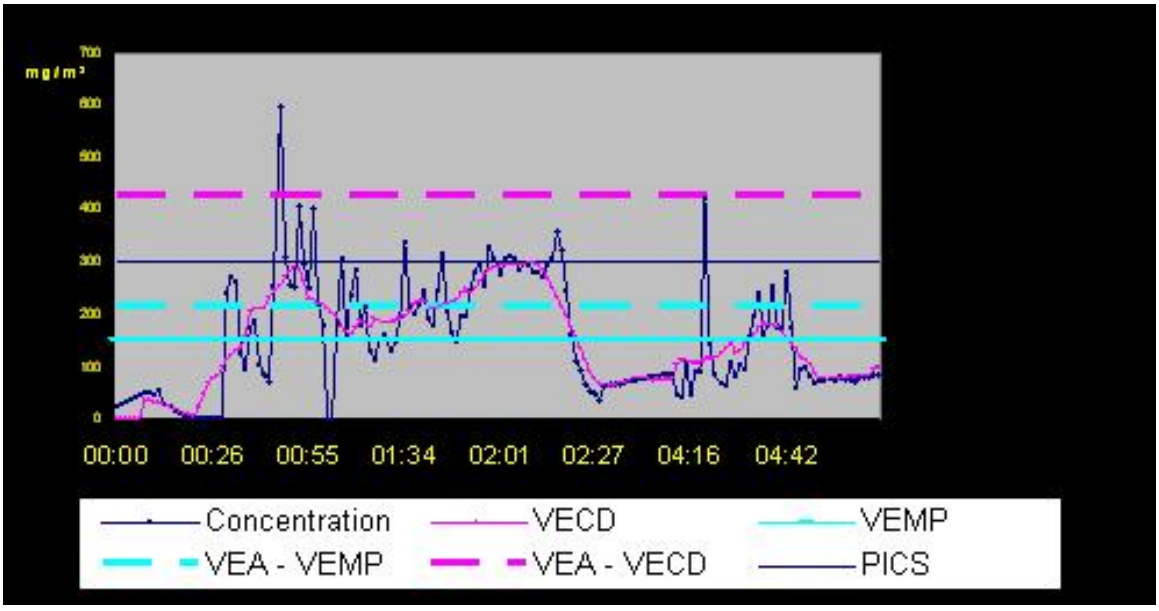
Candidate selection criteria

Criteria related to the exposure level (Exposed worker – without peaks)



Candidate selection criteria

Criteria related to the exposure level (Exposed workers – with peaks)



Candidate selection criteria

Other criteria

- Be a man in good health
- Be under 60 years of age
- Not be dependent on alcohol or drugs
- Be employed by the establishment for at least six months
- Not be and not having been exposed to other neurotoxic substances (solvents, etc.)
- Not wear respiratory protection during his usual work

Recruitment of establishments

Needs

- Within the framework of the study
  - 40 exposed workers with peaks,
  - 40 exposed workers without peaks, and
  - 40 controls (unexposed) from the same establishments as the exposed workers.

  Therefore, a total of 120 people whose styrene exposure must be characterized

Recruitment of establishments

State of the situation

- In the Québec reinforced plastics sector there are approximately
  - 58 establishments with 20 or more production workers for a total of 4350
  - 77 establishments with fewer than 20 production workers for a total of 470
- **In eight months, we recruited only 2 establishments and approximately 5 workers.**

CSST Schedule I committee

- The committee's mandate?
  Revising the PEVs of regulated contaminants in Québec.
- Composition of Schedule I committee?
  Committee mandated by the CSST's board of directors consisting of employer and labour representatives.

CSST Schedule I committee

- Hazardous materials studied in 2004?
  5 to 6 substances including styrene
- Current orientation of the committee regarding PEVs of styrene?
  Without reaching 20 ppm (85mg/m³) for the PEV-TWAEV, a lower value can be anticipated.

Research project of the Styrene Information and Research Center

PEV of styrene in ppm

Organization	Permissible exposure value		
	TWAEV	STEV	Ceiling
Québec	50	100	
ACGIH	20	40	
United States	100		200
Germany	20	40	
Sweden	20*	50	
*Any new establishment must aim for 10 ppm			

Research project of the Styrene Information and Research Center

Objectives

- Establish a dose-effect relationship for styrene up to 213 mg/m³ (50 ppm)
- Characterize the workers' exposure
- Measure the acute vs chronic effects
  - Neurobehavioural effects
  - Vision
  - Hearing
- Collect historical exposure data (5 years)

Research project of the Styrene Information and Research Center

Recruitment of establishments

- Establishments in Ontario
  Representative: **C**anadian **P**lastics **I**ndustry **A**ssociation

- Establishments in Québec
  Representative: **R**egroupement des **I**ndustries des **C**omposites du **Q**uébec?
  Canadian **P**lastics **I**ndustry **A**ssociation?

Conclusion

- Advantages for the establishments
  - Exposure mapping
  - Recommendations relating to exposure control

- Disadvantages for the establishments
  - Intrusion into operations during measurements
  - Follow-up to be provided when exposure above the current PEVs is identified

APPENDIX IX: THE RICQ BOARD OF DIRECTORS' RECOMMENDATIONS AT ITS MEETING HELD MAY 4, 2004, REGARDING ITS MEMBERS' PARTICIPATION IN THE STYRENE RESEARCH PROJECT[2]

St-Jérôme, May 4, 2004

Mr. Charles Beaudry, MSc[2]
Research officer
Department of environmental and occupational health
Université de Montréal

SUBJECT: Study Phase II

Mr. Beaudry,

At its last meeting of the Board of Directors held April 30th, the RICQ, after having read the minutes of the information meeting held April 14, 2004, relating to your research project entitled: "*Effects of concentration peaks on styrene neurotoxicity in the fibreglass reinforced plastics industry – Phase II,"* decided to collaborate in your study and to encourage the participation of its members who could be interested in the subject.

This endorsement of your project's objectives is concrete action towards research on styrene and its goal is a two-way exchange of information so as to improve the environmental conditions to which our employees are exposed.

To properly understand the context that allowed this decision to be reached, enclosed is a copy of the Board of Directors' resolution.

I hope that this meets your expectations and look forward to working together in carrying out this project.

Yours truly,

Claudine Leblanc
Administrative assistant
Duly mandated by
The RICQ Board of Directors

Encl.

[2] Translation of original French letter

Resolution adopted unanimously at the Board of Directors' meeting of April 30, 2004

Resolution

Whereas	Dr. Vyskocil's team came to present his Phase II research project, entitled "Effects of concentration peaks on styrene neurotoxicity in the fibreglass reinforced plastics industry – Phase II," on April 14th last;
Whereas	clarifications were provided for different questions previously asked by the RICQ;
Whereas	the concept of "PEAK" was clearly established, as well as the fact that at no time does it refer to an exceedence of the currently applicable control standard;
Whereas	in carrying out the project, participating employees will not be required to be exposed to non-regulatory excesses of styrene;
Whereas	during the project, the employees will work within the exposure limits permitted by current regulations;
Whereas	the additional costs for participating manpower will be assumed by the research team (overtime after their work in the plant);
Whereas	the research team assures us of its support for any ambiguity in interpretation of the standards in force with respect to the CSST that could result during the project;
Whereas	the RICQ will be regularly and prioritarily informed about the evolution in the work and/or any anomaly that could be noted in-plant;
Whereas	this project will be completely carried out in the strictest confidentiality, with respect to the participants as well as the results;

It is therefore proposed by Mr. Germain Bélanger and seconded by Mr. Pierre Larivière to participate actively in this study.

APPENDIX X: STEPS UNDERTAKEN WITH THE RICQ FOLLOWING SUSPENSION OF THE RESEARCH PROJECT[3]

May 9, 2006

Mr. François Chevarie
President
Regroupement des industries des composites du Québec (RICQ)
764, rue Bouchard – Lavallée
St-Jérôme (Québec) J7Z 7B8

Mr. Chevarie,

On behalf of the participants from the *Université de Montréal* at this morning's meeting with RICQ representatives, we want to thank you for your visit to the university to discuss the ongoing research project on styrene, a project funded by the *Institut de recherche Robert-Sauvé en santé et en sécurité du travail* du Québec (IRSST). In addition to yourself, the RICQ members who were present were Bernard Marcoux, Pierre Larivière, Louis Dionne and Jean-Guy Picard. From the *Université de Montréal*, Elmira Aliyeva and France Gagnon as well as Ross Thuot, Charles Beaudry, Adolf Vyskocil and Claude Viau were present.

The RICQ members had the opportunity to make us better informed about the RICQ's objectives and mode of operation. Your organization promotes the exchange of information mainly involving the prevention of the hazards associated with exposure to the chemical substances used in your industry. However, each member is committed to the absolute confidentiality of all information of a commercial nature about the company that he represents, even with other members of the RICQ.

On our side, our interest is focused first on the science of hygiene and toxicology and on application of the knowledge in these fields to the prevention of potentially harmful effects of chemical substances. Therefore, any research project is normally the subject of scientific and sometimes vulgarized publications on the observed results and on the conclusions that we draw from them. However, with the clarifications that you shared with us this morning, we have no hesitation making the commitment not to divulge any information that could identify any of the companies participating in the research project or any information of a commercial nature that could adversely affect this company. This confidentiality of information will be respected in all forms of communication, written as well as verbal, during the presentation of conferences, for example. Regarding the sensitive aspects, we will ask the participating industries to help us formulate the information about the necessary description of the work methods to be linked to the observations on styrene concentrations in the air in such a way that does not betray this confidentiality, while allowing us to communicate the useful information for achieving the objectives of the research project.

[3] Translation of original French letter

We hope that this is consistent with our verbal agreement of this morning. On behalf of all the university's participants in this project, we remain
Yours sincerely,

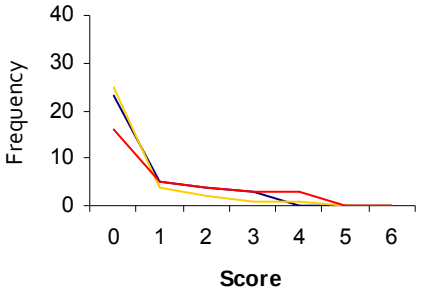
Adolf Vyskocil, associate professor and project director

Claude Viau, full professor and project co-director

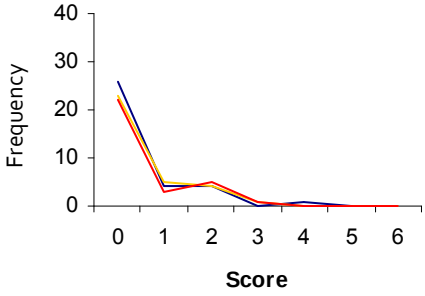
Charles Beaudry, research officer

APPENDIX XI: GRAPHICAL REPRESENTATION OF THE RELATIONSHIP BETWEEN STYRENE EXPOSURE IN THE THREE GROUPS AND THE SYMPTOM SCORES OBTAINED

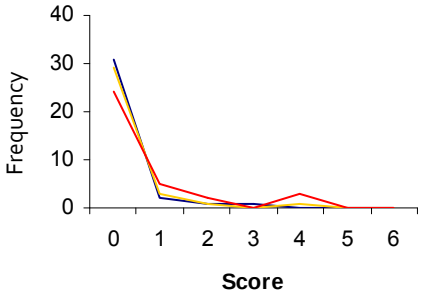
Headache



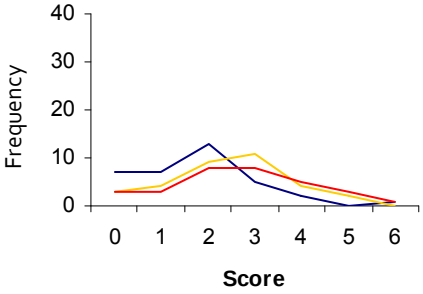
Dizziness



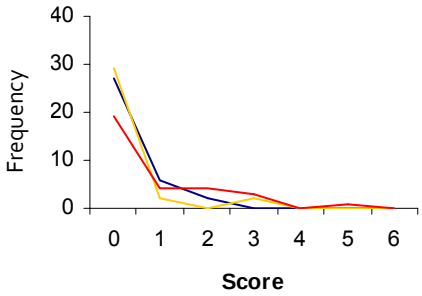
Nausea



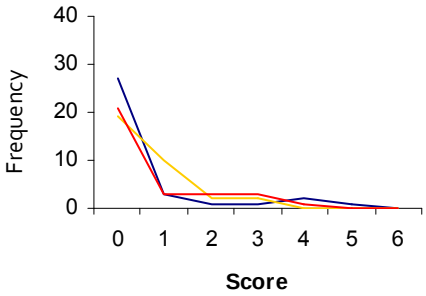
Fatigue



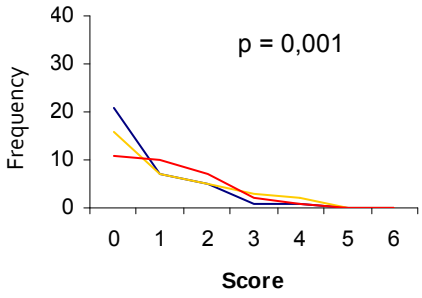
Chest pressure



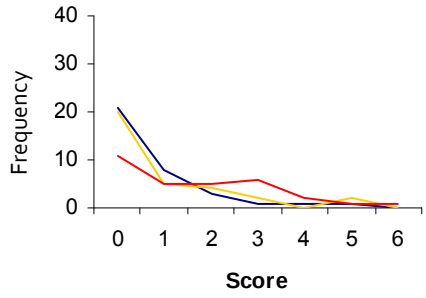
Cough



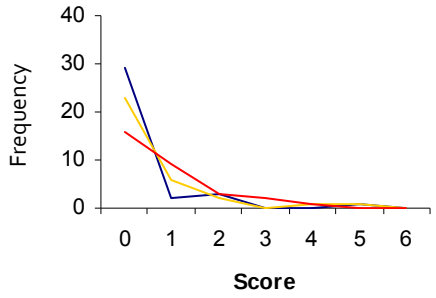
Shortness of breath



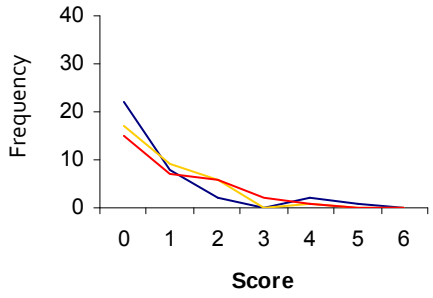
Eye irritation



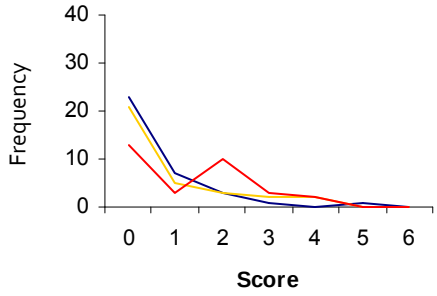
Eye discharge



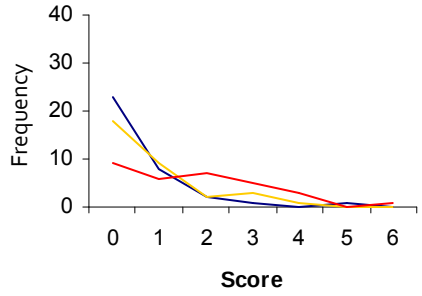
Blurred vision



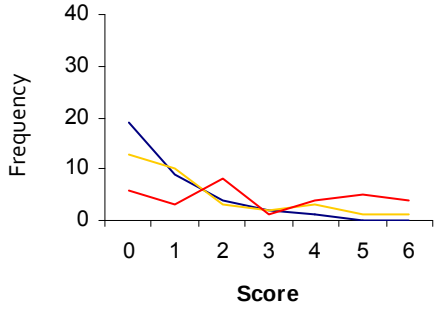
Nasal irritation



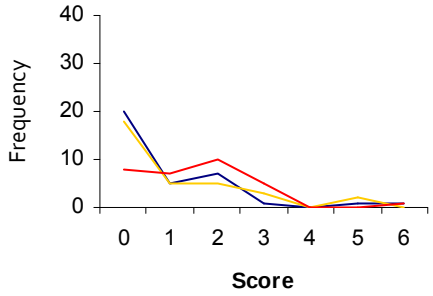
Nasal discharge (runny nose)



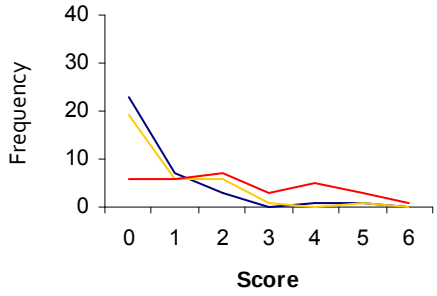
Impression of unpleasant odours



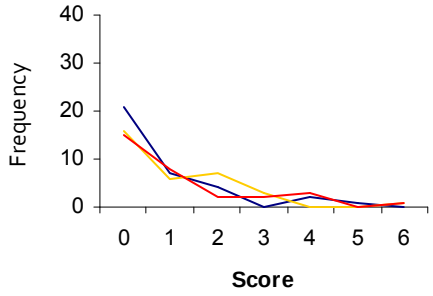
Throat irritation

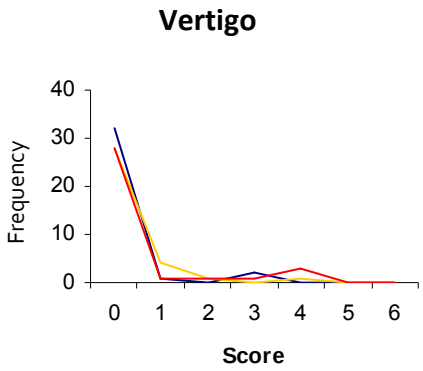


Impression of unpleasant taste



Skin irritation




Vertigo
Frequency
40
30
20
10
0
0 1 2 3 4 5 6
Score