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Study of the neurobehavioural toxicity of styrene at low levels of exposure

D. Jégaden[1], D. Amann[2], J. F. Simon[1], M. Habault[2], B. Legoux[1], and P. Galopin[1]

[1]Service de Médecine du Travail, DCN Lorient, Délégation Générale pour l'Armement, F-56998 Lorient Naval, France
[2]Service de Psychologie Appliquée, Marine Nationale, C.I.N. Saint-Mandrier, F-83800 Toulon Naval, France

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Summary. Thirty workers in a dockyard exposed to concentrations of styrene lower than the TLV-TWA of 50 ppm and 30 control workers not subject to exposure but employed by the same company were subjected to three psychometric tests on one Monday morning and evening. The results were usually better in the evening than in the morning in both groups, which proves the lack of acute intoxication at the end of the day at this level of exposure. On the other hand, all of the tests conducted on the exposed subjects are significantly less good than those on the controls. The results suggest the existence of minor but significant organic mental disorders in the subjects exposed to a mean dose of 30 ppm in this study. These results are inconsistent with those of several recent studies. The advisability of lowering the TLV of 50 ppm in discussed.

Key words: Styrene – Dockyard workers – Neurotoxicity

Introduction

The neurotoxicity of styrene is recognized in the case of acute, severe intoxications. On the other hand, whether neurotoxic effects occur following low levels of exposure is still a matter of conjecture, especially when the concentrations of this solvent are lower than the TLV-TWA value of 50 ppm presently fixed by the American Conference of Governmental Industrial Hygienist's Committee (ACGIH) and by the French Ministry of Work. The difference between an acute or subacute attack and chronic neurotoxicity has, moreover, not been clearly defined at these moderate levels of exposure.

No neurotoxic effect was demonstrated by Gottel et al. (1972), Edling and Ekberg (1985) or Triebig et al. (1989) for concentrations of styrene lower than 100 ppm. On the other hand, Lindström et al. (1978), Mutti et al. (1984) and Flodin et al. (1989) describe anomalies in psychometric tests after exposure to doses lower than 50 ppm and, sometimes, lower than 25 ppm. Nonetheless, the results of these latter studies are not always in agreement.

The question as to whether neurotoxicity is really incurred by exposure to styrene at levels equal to or less than 50 ppm remains unanswered and the discussion as to whether this level for the TLV should be maintained or lowered is a topical one. The aim of the study presented here is to contribute to finding an answer to this question.

Correspondence to: D. Jégaden

Materials and methods

The study was conducted on moulders employed by a large construction company in a French naval dockyard. The material worked with is a glass polyester resin composite, the solvent and copolymer for which is styrene. The work of the moulders consisted in impregnating, by successive layers, glass fabrics with tetrahydrophthalic resin solubilized in styrene. These moulders occupied similar work stations throughout their professional life. Styrene is by far the most used solvent. Methylene chloride is used to clean tools, but for the most part with a closed circuit machine. The concentrations of methylene chloride within the workshop are insignificant.

The control subjects, recruited from other traditional workshops of the company, were carefully matched with the moulders with respect to ethnic group, sex, age and intellectual and sociocultural level. They all worked in a sonic environment similar to that of the moulders since noise might possibly be a source of confusion.

Alcoholic subjects as defined in the DSM III and subjects who showed hepatic disorders, those taking medicines likely to adversely affect vigilance, control subjects exposed to solvents and moulders undergoing too slight an exposure to styrene on the day of the test (exposure less than 4 ppm) were all excluded from the study.

Thirty moulders with a mean age of 28 years (± 6 years) who had exercised their profession for a mean of 5 years (± 4.5 years) and 30 control subjects of the same sex and mean age were finally selected. Eleven moulders had a short length of service (1 year); nine had a medium length of service (ranging from 2 to 5 years) and ten had a long length of service (ranging from 9 to 14 years).

The protocol adopted was the following:

1. The investigation was performed on a Monday, after 2 days without exposure.

2. Exposure of the moulders to styrene during the day of the investigation was quantified by:
   a) Determination of the metabolites of styrene (mandelic acid and phenylglyoxylic acid) in the urine at the beginning of the shift in the morning (0800 hours) and in the evening at the end of the exposure (1600 hours). These determinations were performed by means of gas chromatography. The results were related to creatinine.
   b) Each moulder was equipped with a gas badge throughout the day from 0800 to 1600 hours.
   c) The atmospheric concentration of styrene was measured continuously at each work station by probes connected to an atomic mass spectrometer.
3. The mean level of exposure of the dockyard workforce over a long period was estimated by calculation of the mean of 254 determinations of the styrene urinary metabolites made during 43 weeks of the year 1990 at a frequency of one determination per week, performed each Thursday evening (maximal impregnation) on six workmen selected at random each week.
4. Finally, the moulders and the control subjects were subjected to a battery of psychometric tests before the beginning of work and at the end of the working day. These tests were performed on a microcomputer with software developed by the Department of Applied Psychology of the French navy. The repetition of the testing on the same day was taken into account and the development of the tests in two strictly parallel forms made it possible to limit the bias. Three tests were selected, the first two testing reflex reactions and the third mental reactions:
   a) The first test is a simple test of visual reactions: A signal is presented in the middle of the screen. As soon as he sees it appear, the subject is required to press a key. Thirty stimulations were programmed.
   b) The second test is a selection test of visual reactions: A signal is shown randomly at the top or the bottom of the screen. If this signal appears at the top of the screen, the subject is required to press a key. If it appears at the bottom, he is required to do nothing. Again, 30 stimulations were programmed.
   c) The third test is a test of the memorization of numbers. Sequences of numbers of increasing length (from two to nine digits) appear on the screen. At the end of each sequence, the subject is asked to recopy on a piece of paper the sequence viewed on the screen. One set of sequences must be given in the order shown, another in the reverse order. This last test, commonly called "digit span", is derived from the memory test of numbers given in the Wechsler Adult Intelligence Scale.

   All of the tests were conducted by the same experimenter, were given in the same order and in accordance with the modalities prescribed by a pre-established instruction manual. The treatment of the data involved the comparison of the means of the reaction times and the means of their scatter expressed in seconds in the case of the first two tests and the comparison of the means of the scores obtained in the third test. Student's *t*-test was used after verification of the normality of the distribution.

Results

The levels of exposure to and impregnation of styrene

The characteristics of exposure to styrene throughout the day of the tests were:

1. A mean exposure to styrene of 22.68 ppm with extremes of 4 and 55 ppm.
2. A mean of the determinations of the urinary metabolites (mandelic acid and phenylglyoxylic acid) corresponding to 37.6 mg/g creatinine in the morning with extremes of 0 and 165 mg/g creatinine (only seven moulders had none). In the evening, the mean of the determinations of the metabolites had increased to 574.8 mg/g creatinine, the extreme values being 90 and 2180 mg/g creatinine.

There was a significant correlation between the value for the metabolites in the evening and the level of exposure to styrene during the day ($r = 0.73$, $P < 0.001$).

The results of the 254 determinations of the urinary metabolites made over 43 weeks gave a mean of 721.85 mg/g creatinine (confidence interval: 610–833 mg/g creatinine). This value corresponds approximately to a mean exposure of 30 ppm of styrene; 20% of the determinations corresponded to values higher than 50 ppm.

The Psychometric tests

The control group was set up by comparison with a reference group in the Department of Applied Psychology of the Navy.

In the test of simple visual reactions, the control group and the exposed group improved their reaction times and their scatter during the repeat of the test in the evening compared with the results of the test in the morning (Table 1). This improvement was not significant, except for the scatter in the case of the controls; it was, however, less marked in the case of the exposed subjects than for the controls. On the other hand, the exposed subjects performed significantly less well than the control group in the morning ($P < 0.02$) and in the evening ($P < 0.01$).

The results were similar in the selection test of visual reactions (Table 2). In this test, the analysis of the stimulus increases slightly the mean response time and the scatter of the individual results. The improvement in the results between the morning and the evening was similar, i.e. not significantly different, in the two groups. However, the exposed group always showed significantly longer ($P < 0.01$) reaction times than the controls in both the morning and the evening. These two tests thus clearly demonstrated that the subjects exposed to styrene reacted more slowly than the controls, although there was also no deterioration in the results at the end of the day.

In the test of the memorization of numbers, the control and exposed groups improved their scores in both the forward and backward, directions between the morning and the evening (Table 3). Here, too, the scores achieved by the exposed subjects were significantly less good than those of the controls, even in the morning.

The subjects exposed to styrene thus exhibited a constant deterioration in feats of memory compared with the controls, but no deterioration was observable during the course of the day.

For each test, a differential study according to the duration of the exposure and the level of exposure on the day of the tests was performed by dividing the exposed population into three equal groups and by comparing the results of the individuals of the two extreme groups for each of the two parameters. No significant

Table 1. Results of the test of simple visual reactions (test 1; times in seconds)

	Controls		Exposed		Level of significance
	Mean	Standard deviation	Mean	Standard deviation	
Morning					
Reaction time	0.27	0.04	0.29	0.04	$P < 0.02$
Scatter of reaction times	0.07	0.03	0.08	0.03	NS
Evening					
Reaction time	0.25	0.04	0.29	0.03	$P < 0.01$
Scatter of reaction times	0.05	0.01	0.07	0.02	$P < 0.01$

NS, Non-significant

Table 2. Results of the selection test of visual reactions (test 2; times in seconds)

	Controls		Exposed		Level of significance
	Mean	Standard deviation	Mean	Standard deviation	
Morning					
Reaction time	0.32	0.05	0.37	0.06	$P < 0.01$
Scatter of reaction times	0.07	0.03	0.08	0.03	NS
Evening					
Reaction time	0.30	0.04	0.35	0.05	$P < 0.01$
Scatter of reaction times	0.06	0.02	0.07	0.03	NS

NS, Non-significant

Table 3. Results of the test of the memorization of numbers (test 3; scores)[a]

	Controls		Exposed		Level of significance
	Mean	Standard deviation	Mean	Standard deviation	
Morning					
Order shown	4.80	1.15	4.20	1.42	NS
Reverse order	5.06	1.31	3.16	1.53	$P < 0.01$
Evening					
Order shown	5.43	1.19	4.43	1.25	$P < 0.01$
Reverse order	5.63	1.19	3.66	1.39	$P < 0.01$

NS, Non-significant

[a] In none of the tests, as detailed in Tables 1–3, were the differences between the morning results and the evening results significant for the exposed group or the controlled group

Table 4. Comparison of test results between subjects submitted to a short and to a long period of exposure to styrene

Test			1 year's exposure	8–14 years' exposure	Level of significance
Test 1 (seconds)	A		0.30 ± 0.05	0.29 ± 0.05	NS
	B		0.30 ± 0.04	0.28 ± 0.03	NS
Test 2 (seconds)	A		0.36 ± 0.06	0.37 ± 0.07	NS
	B		0.34 ± 0.03	0.37 ± 0.07	NS
Test 3 (scores)	A	O.S.	5 ± 1.6	4.1 ± 0.9	NS
		R.O.	3.8 ± 1.8	2.9 ± 1.4	NS
	B	O.S.	4.7 ± 1.5	4.9 ± 0.7	NS
		R.O.	4 ± 1.7	3.3 ± 1.2	NS

A, Morning; B, evening; O.S., order shown; R.O., reverse order

difference was found (Table 4). Thus, there is apparently no worsening of the effect as a function of the time of the exposure to styrene nor is there an obvious dose-response relationship, but the small number of subjects compared does not permit reliable conclusions to be drawn.

Discussion

The results of the tests conducted in the evening were always at least as good as, and even slightly better than, those done in the morning for both the controls and the subjects exposed to styrene. This is explained by a learning effect due to the proximity of the tests in time and the similarity of the task. The absence of worsening of the scores in the three tests in the evening compared with the morning in the exposed subjects also reflects, in our opinion, the lack of acute neurotoxicity at levels of exposure less than 50 ppm (TLV).

In our study, we paid particular attention to the exposure-impregnation relationship of the styrene, and we found a significant correlation between exposure and elimination of metabolites, quite similar to the results of Ikeda et al. (1982).

The risk of a deterioration in vigilance at the end of the working day thus seems to us to be non-existent at these levels of exposure, and a considerably greater exposure is needed to cause the tendency to sleepiness noticed from time to time in these workmen after a day's work. In this connection, Cherry et al. (1980), who used a methodology similar to ours, found a greater frequency of disorders of mood and fatiguability after a day's work in exposed subjects compared with controls, but for an exposure to styrene higher than ours, of the order of 92 ppm.

On the other hand, the modest but significant fall noted in the performances of the exposed subjects in all of the test, in particular those conducted on Monday morning, may reflect the existence of chronic neurotoxicity. Such chronic neurotoxicity affects psychomotor performance and cognitive functions, especially short-term memory, also called working memory (Van Der Linden 1989). It is evident if, in the test of the memorization of numbers, the change in the score obtained for the set in the reverse order is compared with that for the set in the order given obtained immediately before (Fig. 1). The disturbances are more marked for memorization in the reverse order in the subjects exposed to styrene, perhaps reflecting a deficiency at the level of the strategy of development of mental patterns.

Our results are in agreement with those of several studies. Lindström et al. (1978), Harkonen and co-workers (Harkonen 1977; Harkonen et al. 1978) and Mackay and Kelman (1986) found impairments in psychometric tests in subjects exposed to concentrations of styrene of the order of 40–50 ppm. Mutti et al. (1984) detected a diminution in verbal memory in subjects exposed to concentrations higher than 25 ppm, but an impairment in logical memory and visuomotor functions only for concentrations higher than 50 ppm. Flodin et al. (1989) recorded a manual dexterity disorder at very low exposures (5–10 ppm).

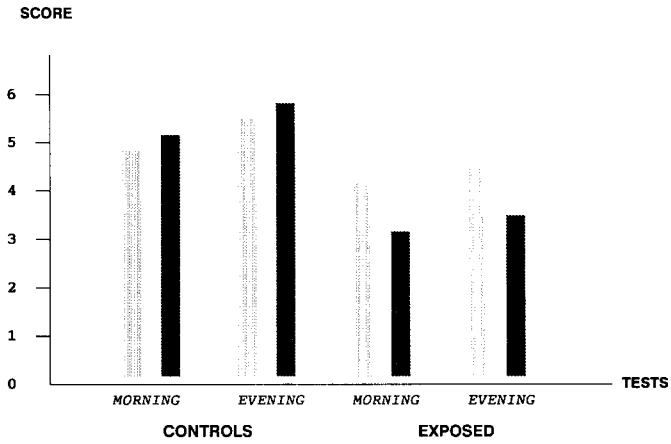

Fig. 1. Comparison of the changes in score on the test of the memorization of numbers in the order shown and in the reverse order by the controls and by the subjects exposed to styrene. ▨ Order shown; ■ reverse order

By contrast, Gottel et al. (1972) failed to observe any increase in the simple reaction time for an exposure less than 150 ppm in 17 exposed subjects, as did Edling and Ekberg (1985) for 12 workers exposed to 25 ppm of styrene. Similarly, Triebig et al. (1989) using a battery of 12 psychometric tests, detected no significant difference in 36 subjects exposed to concentrations varying from 3 to 251 ppm with a median of 18 ppm.

In spite of these contradictory results, it nonetheless seems that a consensus exists today which admits the possibility of organic mental disorders occurring as a result of exposure to relatively low levels of styrene, and in any case lower than the TLV of 50 ppm. Our study confirms this point of view. For all that, must the TLV of 50 ppm be considered unacceptable and is it necessary to ask for a reduction of the latter to 25 ppm, as is already practised in certain Scandinavian countries and Germany? This is debatable in view of the fact that these anomalies remain minor and do not appear to get worse with time. The serious question is whether the anomalies are acceptable or not to the individual and the community. If these disorders actually occur within a short period of exposure (less than 1 year), it seems that they do not cause the mental state to deteriorate progressively with age length of exposure.

The study which we have conducted shows that at levels of exposure to styrene lower than the TLV of 50 ppm, there are no acute signs of neurotoxicity at the end of the day which could impair vigilance. On the other hand, it is clearly evident that the workers exhibit minor but significant organic mental disorders at these levels of exposure, as reflected in a constant and consistent decline in the performance of psychometric tests compared with carefully selected matching controls.

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