

Exposure-response relationship between styrene exposure and central nervous functions

by HANNU HÄRKÖNEN, M.D., KARI LINDSTRÖM, Ph.L., ANNA MARIA SEPPÄLÄINEN, M.D., SISKI ASP, M.Pol.Sc., and SVEN HERNBERG, M.D.¹

HÄRKÖNEN, H., LINDSTRÖM, K., SEPPÄLÄINEN, A. M., ASP, S. and HERNBERG, S. Exposure-response relationship between styrene exposure and central nervous functions. *Scand. j. work environ. & health* 4 (1978) 53—59. For the study of the relationship between styrene exposure and symptoms and signs of central nervous dysfunctions, 98 male workers occupationally exposed to styrene were given clinical, neurophysiological and psychological examinations; also a symptom survey was made. Urinary mandelic acid concentrations, measured once a week during five consecutive weeks, were used to express the exposure intensity. Different unexposed groups were used for reference. No exposure-response relationship was observed between symptoms of ill health and the urinary mandelic acid concentration, although the exposed group as a whole expressed significantly more symptoms than the reference group. The occurrence of abnormal electroencephalograms was about 10 % in the group of workers with mandelic acid concentrations below 700 mg/l, but it was 30 % among those whose mandelic acid concentration exceeded 700 mg/l, a level corresponding to the 8-h time-weighted average (TWA) of styrene exposure of about 30 ppm. With regard to psychological functions, the first change in visuomotor accuracy became discernible when the urinary mandelic acid concentration exceeded 800 mg/l. A more pronounced decrement appeared in both visuomotor accuracy and psychomotor performance when the mandelic acid concentration exceeded 1,200 mg/l, which corresponds to an 8-h TWA of styrene exposure of about 55 ppm.

Key words: electroencephalographic findings, mandelic acid excretion, psychological impairment, safety standards, styrene exposure.

Styrene is one of the most widely used raw materials for plastics. For example, in the U.S.A. about two million tons were produced in 1975 (3). Most of the styrene synthesized is used in the production of polystyrene plastics and styrene copolymers. The unsaturated polystyrene plastic industry is another major consumer; it uses styrene as a solvent and as a cross-linking agent for polyester resins. Especially this type of production causes signif-

icant exposure to the workers involved. It has been estimated that about one-tenth of the styrene used evaporates into the air of the workroom during the curing process (12). Peak concentrations during laminating may even exceed 500 ppm (4).

Styrene is known to be toxic to the central nervous system. In addition, it is an irritant. These effects form the basis for the current threshold limit value (TLV) of 100 ppm (1, 2) that has been derived by the American Conference of Governmental Industrial Hygienists (ACGIH), mainly from short-time human experiments in exposure chambers (5, 23). The few studies published concerning the effects of occupational styrene exposure have not

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yet been utilized in standard setting (7, 8, 18).

The use of field studies in the construction of exposure-response curves, which are obligatory for meaningful standards, is highly facilitated whenever good biological exposure tests are available. A team from our institute showed earlier that a strong positive correlation ($r = 0.87$) prevailed between the 8-h time-weighted average (TWA) of the concentration of styrene in the breathing zone of laminating workers and urinary mandelic acid concentration adjusted to a constant specific gravity of 1.018 (6). This observation rendered a more extensive field study feasible in that determination of urinary mandelic acid concentration could replace time-consuming air samplings as the measure of exposure. The survey focused on exposure levels at or below the current ACGIH TLV of 100 ppm, and it comprised a symptom questionnaire and clinical, neurophysiological and psychological examinations. The results of these examinations have been reported earlier (10, 16, 22). Our intention is to synthesize the findings and relate them to the estimated TWA exposure in order to provide exposure-response data for the documentation of a safety norm for occupational styrene exposure.

MATERIALS AND METHODS

The exposed subjects, the reference groups, and the methods used have been described in detail in earlier publications (16, 22). In summary, the exposed subjects comprised 98 male laminating workers from 24 plants manufacturing reinforced polyester plastic products. Their median age was 28 years (range 16–54 years), and the duration of their exposure varied from 0.5 to 14 years (median 5.1 years). Urinary mandelic acid concentrations were used as the measure of current exposure (6). The urine samples were collected at the end of the workday from every subject once a week, on consecutive weekdays, for five weeks prior to the clinical examination. The arithmetic mean of the five determinations was used for the estimation of the intensity of the individual exposure. This mean varied from 7 to 4,715 mg/l (median 808 mg/l).

The various effects were studied in reference to different exposure categories, and the validity score of each effect was calculated according to Zielhuis et al. (26).

RESULTS

Earlier studies

According to the symptom survey, the exposed group complained statistically significantly more often of tiredness, concentration difficulties, and irritation symptoms, but no single symptom nor the total symptom score correlated with the mean concentration of mandelic acid in urine (10).

The electroencephalograms (EEGs) were interpreted according to usual clinical criteria (13). Slow wave activity was considered abnormal only if it appeared on several occasions in the waking record. Rare paroxysmal abnormalities consisted of bilateral spike and wave discharges. The overall prevalence of abnormal EEGs was 24 % (23 out of 96), which was higher than that expected for the general population, as judged from EEG literature. An increased prevalence of abnormal EEGs (about 30 %) was found for the subjects whose urinary mandelic acid concentration exceeded 700 mg/l (4.9 mmol), while the number of abnormal EEGs among the subjects with less exposure (below 700 mg/l) was not above that found in the general population (22).

Group comparisons and a stepwise multiple regression analysis showed that, of the psychological functions measured, visuomotor inaccuracy and lowered psychomotor performance were related to a high mean concentration of mandelic acid in urine. Reversals in the Symmetry Drawing test, form level of straight lines in the Mira Test, and errors and omitted reactions in the Bourdon-Wiersma Vigilance Test were the variables which showed a statistically significant relationship to the mean mandelic acid concentration (16). None of these variables had a statistically significant correlation with age.

Intellectual functions were not influenced by styrene exposure. Preexposure intelligence levels could be evaluated from test results obtained from the files of the

Finnish army. The intellectual level of the exposed group was not found to differ from the average level of the general Finnish population, either before or after exposure (15).

Exposure-response relationship

The proportions of normal and abnormal EEGs in different mandelic acid concentration groups are shown in fig. 1. A notable change in the proportions occurred as soon as the mandelic acid concentration exceeded 700 mg/l. The validity score and the chi-square test had their maximal values at this cut-off point (validity = 1.28, $\chi^2 = 4.60$, $p < 0.05$). At the level of 800 mg/l (5.6 mmol) the proportion of abnormal EEGs reached a plateau (table 1).

In order to define the threshold exposure level for a deterioration in psychological functions, we computed the sensitivity and specificity (26) of the most relevant tests. The procedure required dichotomization of both the psychological test variables in question and the exposure variable. For two psychological test variables, Symmetry Drawing and the Mira Test, the value equal to the mean + 1 SD in the reference group was chosen as a cut-off point. The number of persons with functions classified as poorer than the mean + 2 SD was too small for statistical analysis. For the Bourdon-Wiersma test no data from the reference group were available; therefore the cut-off point was chosen from the exposed group itself in a

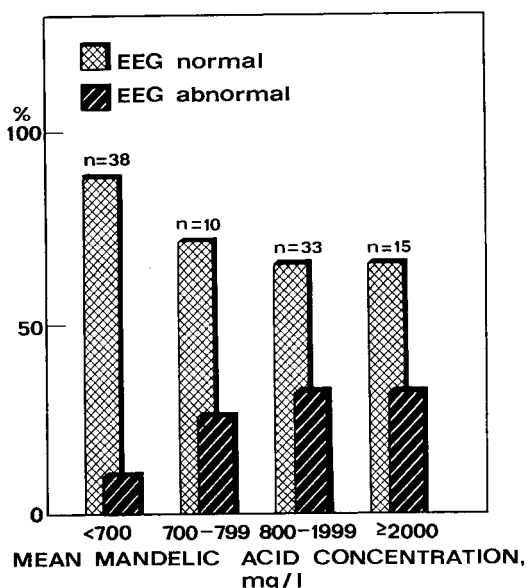


Fig. 1. Relative frequency of the normal and abnormal electroencephalograms of the exposed subjects according to the subjects' urinary mandelic acid concentrations.

similar manner. Calculations were then performed from fourfold contingency tables with cut-off points for mandelic acid concentrations of 800, 1,200, 1,600 and 2,000 mg/l, so that the maximal validity of the prediction of psychological performance by mandelic acid concentration (sensitivity + specificity) could be defined. Also chi-square values were computed for every cut-off point.

Table 1. Distribution of subjects into categories according to their electroencephalographic (EEG) findings and urinary mandelic acid concentrations. The statistical test results and validity calculations are derived from fourfold tables with cut-off points at the lower limit of the mandelic acid concentration category.

Mandelic acid concentration (mg/l)	EEG finding			χ^2	p	Prediction of EEG by mandelic acid concentration		
	Normal	Abnormal	Total			Sensitivity	Specificity	Validity
— 699	34	4	38					
700— 799	7	3	10	5.07	< 0.05	0.83	0.47	1.30
800—1,199	10	7	17	3.66	(< 0.06)	0.70	0.56	1.26
1,200—1,599	8	3	11	0.30	NS	0.39	0.70	1.09
1,600—1,999	4	1	5	0.17	NS	0.26	0.81	1.07
2,000—	10	5	15	0.36	NS	0.22	0.86	1.08
Total	73	23	96					

For the Symmetry Drawing test, which measures visuomotor accuracy, a statistically significant difference was found when the mean mandelic acid concentration exceeded 800 mg/l ($\chi^2 = 4.34$, $p < 0.05$). The significance level of the chi-square increased as the cut-off point for the exposure variable became higher. The validity score and the chi-square value were maximum at a cut-off point of 1,600 mg/l (11.3 mmol) (table 2). The level of the Symmetry Drawing test results, at different levels of mean mandelic acid concentration, is shown in fig. 2. As can be seen, there was a distinct increase in visuomotor disturbance as the mean mandelic acid concentration exceeded 1,600 mg/l. The other variable of visuomotor accuracy, errors and omitted reactions in the Bourdon-Wiersma test showed a reduced level only when the mean mandelic acid concentration was above 2,000 mg/l (14.1 mmol) ($\chi^2 = 5.36$, $p < 0.05$). Also the validity score

had its maximum value at that level (table 2). The results are illustrated in fig. 3.

In psychomotor performance (Mira Test) a statistically significant ($\chi^2 = 4.59$, $p < 0.05$) decline occurred above the mean mandelic acid concentration of 1,200 mg/l (8.5 mmol). The validity calculations supported this result (table 2). Fig. 4 illustrates the relation between psychomotor performance and the exposure level.

No exposure-response relationship emerged for subjective symptoms (10). Even when the mandelic acid concentration was below 700 mg/l there were significantly more symptoms than in the reference group. The frequency of subjective symptoms did not increase as the exposure level increased.

DISCUSSION

The ACGIH TLVs are intended to provide protection from adverse health effects for

Table 2. Distribution of subjects into categories according to their psychological test results and urinary mandelic acid concentrations. The statistical test results and validity calculations are derived from fourfold tables with cut-off points at the lower limit of the mandelic acid concentration category.

Mandelic acid concentration (mg/l)	Performance on psychological test			χ^2	p	Prediction of psychological performance by mandelic acid concentration		
	Better than or equal to cut-off value	Poorer than cut-off value	Total			Sensitivity	Specificity	Validity
<i>Symmetry Drawing</i>								
— 399	20	2	22	.	.	.	.	.
400— 799	19	5	24	3.19	NS ^a	0.92	0.28	1.20
800—1,199	15	3	18	4.35	< 0.05	0.72	0.55	1.27
1,200—1,599	9	3	12	9.26	< 0.01	0.60	0.76	1.36
1,600—1,999	3	2	5	12.98	< 0.001	0.48	0.89	1.37
2,000—	5	10	15	12.84	< 0.001	0.40	0.93	1.33
Total	71	25	96					
<i>Bourdon-Wiersma Vigilance Test</i>								
—1,199	58	8	66	.	.	.	.	.
1,200—1,599	10	2	12	1.76	NS ^a	0.50	0.71	1.21
1,600—1,999	5	—	5	2.30	NS ^a	0.38	0.83	1.21
2,000—	9	6	15	5.36	< 0.05	0.38	0.89	1.27
Total	82	16	98					
<i>Mira Test</i>								
—1,199	54	12	66	.	.	.	.	.
1,200—1,599	9	3	12	4.59	< 0.05	0.52	0.74	1.26
1,600—1,999	3	2	5	6.39	< 0.05	0.40	0.86	1.26
2,000—	7	8	15	5.59	< 0.05	0.32	0.60	1.22
Total	73	25	98					

^a Nonsignificant.

nearly all workers under conditions of continuous exposure during 40 h a week for a working lifetime (2). Because of wide variations in individual susceptibility, exposure of an occasional individual at or even below the TLV may not prevent discomfort, aggravation of a preexisting condition, or even occupational illness (2). Several types of effects can be used as a basis for a TLV, e.g., organic effects, such as carcinogenicity, biochemical effects, such as changes in enzyme activity, and functional effects, such as irritation or changes in sensory and higher nervous functions (11). A WHO Study Group has also stated that a statistically significant effect, as such, is not the same as impairment of health; one should therefore distinguish between effects as such and adverse, i.e., unacceptable, effects. What is regarded as unacceptable (not permissible) is a matter of interpretation and ultimately of choice (25).

In this study we have tried to define the lowest mandelic acid concentration at which statistically significant changes appear in neurophysiological and psychological functions. Based on previous work (6), it is possible to estimate the average exposure level from the concentration of mandelic acid in urine. In fig. 5 the mandelic acid concentration is plotted as the independent variable; the corresponding average styrene concentration can be read from the ordinate. Because of the wider variation of the higher values, the regression equation and the correlation coefficient were calculated with the logarithmic values of both variables. The prevalence of abnormal EEGs exceeded that of the general population starting from a level of 700 mg of mandelic acid per liter of urine. Also the earliest changes in visuomotor inaccuracy became measurable at about the same level. The mandelic acid concentration of 700 mg/l, corresponding to an 8-h TWA styrene exposure of 31 ppm, would therefore indicate the level after which the first changes appeared. More pronounced decrements in visuomotor and psychomotor performance became discernible when the mean mandelic acid concentration exceeded 1,200 mg/l, which corresponds to an 8-h TWA styrene exposure of about 55 ppm (95 % tolerance limits being 25 and 120 ppm). The proportion of abnormal EEGs did not

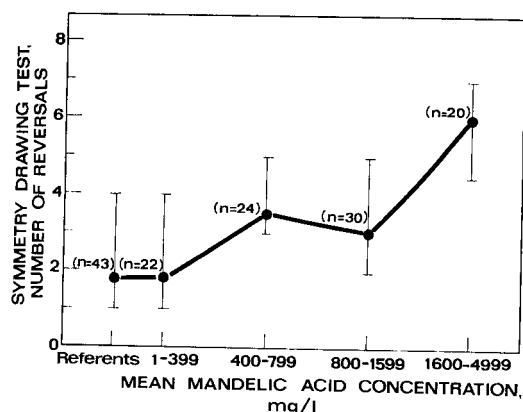


Fig. 2. Median and interquartile range of reversals in the Symmetry Drawing test according to the subjects' urinary mandelic acid concentration.

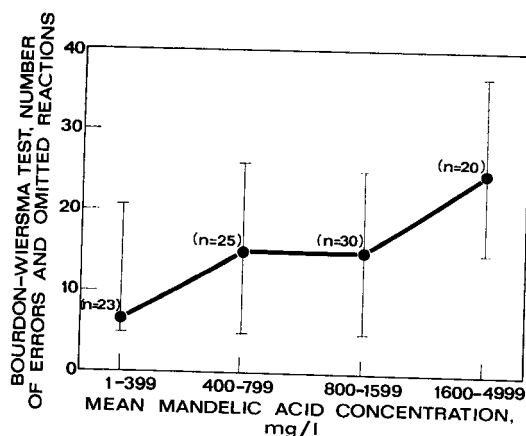


Fig. 3. Median and interquartile range of errors and omitted reactions in the Bourdon-Wiersma Vigilance Test according to the subjects' urinary mandelic acid concentration.

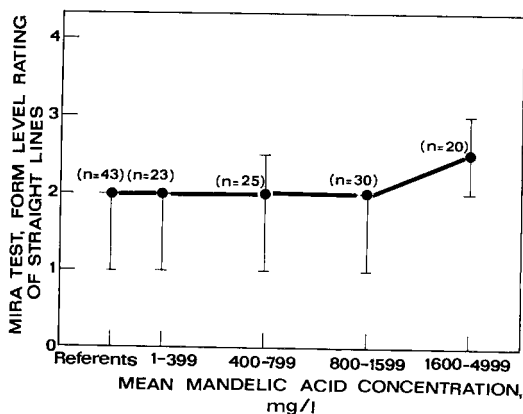


Fig. 4. Median and interquartile range of form level in the Mira Test according to the subjects' urinary mandelic acid concentration.

TWA STYRENE CONCENTRATION, ppm
Log. scale

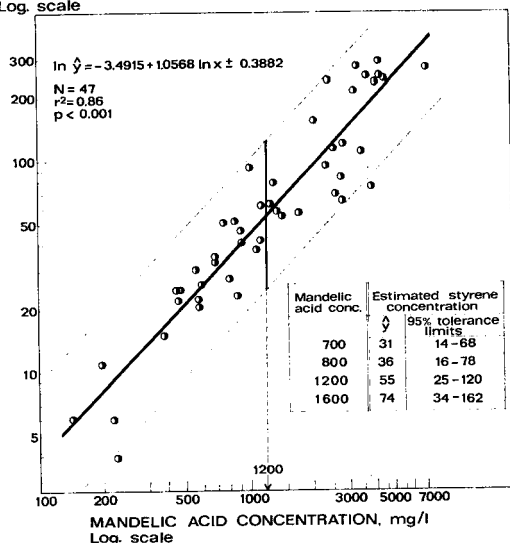


Fig. 5. Correlation between the 8-h time-weighted average (TWA) of styrene exposure and urinary mandelic acid concentration, adjusted to a constant specific gravity of 1.018.

increase further as exposure became more intensive. Hence there emerged an exposure-response relationship between psychological changes and EEG abnormalities on one hand and styrene exposure on the other. However, the psychological changes that occurred were rather mild and were confined to a narrow area of functions, when compared, for example, with those caused by carbon disulfide exposure (9, 16).

The interpretation of these findings in terms of health impairment and work capacity is difficult. Visuomotor accuracy and unimpaired psychomotor performance are no doubt important for demanding operations and a prerequisite for safety at work. Impairment of such functions need not necessarily directly affect the work performance under normal conditions, but they may have an indirect impact by demanding more adaptation and more energy for compensation. An additional difficulty in assessing the significance of changes in behavioral functions is the fact that these functions vary greatly in the general population. However, the aforementioned WHO Study Group (25) stated that the study of early effects on higher nervous functions has attracted in-

creased attention in recent years. The significance of such effects is not yet well established.

Cross-sectional studies in general are influenced by the healthy worker effect, i.e., the most affected workers may have changed jobs, and even retired in extreme cases. Such an effect tends to weaken the measured relationship between exposure and response (14). Hence our results may yield a slightly ameliorated picture of the functional impairment that develops during styrene exposure to TWA concentrations below the current ACGIH TLV of 100 ppm.

To what extent, if any, occasional peak exposures are responsible for the changes cannot be determined from exposure data reflecting only the TWA exposure. (e.g., the concentration of mandelic acid in urine). Likewise, if past exposure levels have differed systematically from the present ones, some bias may have been introduced. Hence conclusions can be drawn only regarding current average exposure levels, not about ceiling values or accumulated past exposure. However, all this reasoning may soon become irrelevant from the point of view of standard setting, because recent studies suggest that central nervous effects may not be the most serious results of styrene exposure.

During the last two years it has become evident that styrene oxide, a metabolite of styrene, is mutagenic (17, 20, 24). Also styrene itself appears to be mutagenic after metabolic activation in *Salmonella* cultures. Mutagenicity tests in general have been found to be indicative of carcinogenicity (21) and these findings have caused concern for styrene. This concern is strengthened by the observation that chromosomal aberrations are unduly prevalent in lymphocytes of workers exposed to styrene (19). So far, direct evidence of carcinogenic effects caused by styrene is lacking, but, if such effects become established, the setting of standards for safe exposure must be regarded from a completely new angle.

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