Neurotoxicology and Teratology. Vol. 12, pp. 665–668. © Pergamon Press plc, 1990. Printed in the U.S.A. 0892-0362/90 $3.00 + .00

Neurobehavioral Effects of Acute Styrene Exposure in Fiberglass Boatbuilders[1]

R. LETZ,*[2] F. C. MAHONEY,* D. L. HERSHMAN,* S. WOSKIE† AND T. J. SMITH†

**Division of Environmental and Occupational Medicine Mount Sinai School of Medicine, New York, NY 10029*
†Department of Family and Community Medicine, University of Massachusetts, Worcester, MA

LETZ, R., F. C. MAHONEY, D. L. HERSHMAN, S. WOSKIE AND T. J. SMITH. *Neurobehavioral effects of acute styrene exposure in fiberglass boatbuilders*. NEUROTOXICOL TERATOL **12**(6) 665–668, 1990.—A field investigation of the effects of acute exposure to styrene among fiberglass boatbuilders was performed. Personal samples of styrene in breathing zone air and postshift urinary mandelic acid were collected for 105 workers exposed and not exposed to styrene in 6 fiberglass boatbuilding companies in New England. Three tests from the computerized Neurobehavioral Evaluation System (NES) were performed by the subjects in the morning before exposure to styrene, near midday, and at the end of the work day. Duration of exposure averaged 2.9 years (SD = 4.6), 8-hour TWA styrene exposure averaged 29.9 ppm (SD = 36.2), and urinary mandelic acid averaged 347 mg/g creatinine (SD = 465). Regression analyses indicated a statistically significant relationship between postshift performance on the Symbol-Digit test and both acute styrene exposure and mandelic acid. Other analyses comparing workers exposed to less than 50 ppm and greater than 50 ppm styrene also showed a significant effect on Symbol-Digit performance. All three NES tests showed test-retest correlation coefficients above .80, and ease of use for collection of neurobehavioral data under field conditions was demonstrated.

Neurobehavioral testing Epidemiologic studies Computerized testing Styrene

WORKERS are exposed to the organic solvent styrene in the lamination of fiberglass. Exposure to styrene at concentrations above 100 ppm is known to have effects on the central nervous system (13). Impaired performance on neurobehavioral tests, as an index of neurotoxicity, has been reported since 1972 (7). There have been several field studies showing neurobehavioral effects using paper and pencil and automated reaction time methods (3, 6, 10, 12).

At the time that the current study was initiated the United States Permissible Exposure Limit (PEL) was 100 ppm for an 8-hour time-weighted average, and the U.S. National Institute for Occupational Safety and Health had recommended a limit of 50 ppm (13). (In February of 1989 the U.S. PEL was lowered to 50 ppm.) The 100 ppm limit was often exceeded, and the 50 ppm limit is regularly exceeded (13). The present study was undertaken to investigate the acute neurobehavioral effects of exposure to styrene in the workplace. Our computerized Neurobehavioral Evaluation System (NES) (1) was designed for field investigation in which repeated testing of individuals is required. Although NES has previously shown utility in detecting effects of acute exposure to intoxicants in humans in the laboratory (8, 9, 11), and of chronic exposure to neurotoxicants in cross-sectional epidemiologic investigations (2,5), additional work was considered necessary to establish whether NES tests were suitably sensitive for use in field testing situations involving repeated testing of individuals.

In the present study three NES tests were administered repeatedly over the course of a work day to fiberglass boatbuilders to investigate the effects of acute exposure to styrene.

METHOD

Subjects

More than 30 fiberglass boatbuilding companies in New England were approached to participate in the study. Management at five fiberglass boatbuilding companies agreed to participate. (One was studied during summer and again during winter-time ventilation conditions for a total of six "companies.") All fiberglass laminators at each company were asked to participate. In addition to laminators, other workers partially exposed or not exposed to styrene were recruited. One hundred eighteen workers

[1]Presented at the 2nd Meeting of the International Neurotoxicology Association, Sitges, Spain, May 22–26, 1989.

[2]Requests for reprints should be addressed to Richard Letz, Ph.D., Division of Environmental and Occupational Medicine, Mount Sinai School of Medicine, 1 Gustave Levy Place, Box 1057, New York, NY 10029.

agreed to participate in the study. All subjects provided informed consent in accordance with institutional guidelines. The companies were compensated for each participant's lost work time. Each subject was sent a letter explaining the results of his test performance and exposure level.

Exposure Assessment

The primary measurement of exposure was 8-hour time-weighted average (TWA) styrene in air obtained with personal monitoring pumps (Dupont). The pump inlet was positioned near the breathing zone, and samples were collected with charcoal tubes. Analysis was via gas chromatography. Urinary mandelic acid (UMA), the major metabolite of styrene, was also measured. A spot urine sample was obtained near the end of workshift from each participant and frozen for later analysis. Samples were analyzed by high pressure liquid chromatography, according to a standard method (14) by a commercial laboratory (ESA Laboratories, Bedford, MA). UMA concentrations are reported as milligrams per gram of urinary creatinine. Finally, the duration in the fiberglass laminating trade (as assessed by questionnaire) was used as an index of chronic styrene exposure.

Neurobehavioral Tests

Three computerized tests from the Neurobehavioral Evaluation System (NES), Version 3.46 were used. They were administered according to a standard procedure, described in the *NES User's Manual* (available from the authors). Briefly, in the *Continuous Performance Test* subjects are asked to press a button in response to a block letter "S" which appears randomly as part of a five minute series of other block letters (B,C,E,A) that flash at the center of the video screen at the rate of one per second. Reaction times in milliseconds to the target stimuli are recorded along with errors of commission and omission. The *Hand-Eye Coordination Test* evaluates manual dexterity and coordination. The participant uses a joystick to trace a large sine wave pattern on the video display. Vertical deviation from the wave pattern is recorded (as root mean square error and mean absolute error). The summary measure was a trimmed mean of the four trials given. The *Symbol-Digit Substitution Test* measures coding skills, attention and concentration. Symbols are matched with the digits 1 through 9 in a "key" at the top of the screen and the participant must enter the digits associated with a row of the symbols in scrambled order below. The response latencies for completing four sets of 9 pairs are recorded, and the preferred summary measure is a trimmed mean time per correct response, i.e., a mean of the two best of the four trials (4).

Testing Protocol

A brief demonstration of the computerized neurobehavioral testing system was given during recruitment to orient the participants to the computerized tests. The NES tests were administered to each participant on three separate occasions (15–20 minutes each) during a single day: early morning, near midday, end of workshift. The subjects were tested 4 at a time in a separate room away from the work area. The computer stations were visually isolated from each other and each participant returned to the same computer station for each testing session. During the final testing session a subjective evaluation of physical symptoms was administered on the computer following the completion of the three NES tests. In addition, a brief interviewer administered questionnaire was completed by each of the subjects to obtain demographic information, the number of alcoholic beverages consumed each week, history of occupational exposure and current job title.

A personal monitoring pump was placed on each participant immediately after the morning testing session and removed immediately before the afternoon testing session. Exhaled breath samples were collected immediately prior to each testing session, but those results are to be reported separately. Urine specimens were collected near the end of the work day.

RESULTS

Demographics

Field studies were conducted at six fiberglass boatbuilding companies, with 8 to 32 subjects tested at each company. Of the 118 subjects tested, 13 were excluded from the data analyses related to exposure according to criteria determined a priori. These included: females (1), grossly incomplete data (withdrawal before completion of the protocol—1), very poor understanding of English instructions (2), history of head trauma (1), management personnel (older, college educated, little exposure—7), self-report of poor effort in performing the neurobehavioral tests (1). Basic demographic and exposure information for the remaining 105 subjects is listed in Table 1. The subjects were generally young with short job tenures, except for employees at Companies 1 and 6. Educational attainment was almost universally high school level.

Exposure

Geometric mean UMA and 8-hour TWA personal styrene exposures are also presented in Table 1. Exposure in the 6 companies varied greatly. Styrene in air and UMA were higher for samples taken during winter ventilation conditions than during summer (Companies 1, 4, 5 and 6 vs. 2 and 3). There were 25 (23%) 8-hour TWA samples with greater than 50 ppm styrene, 9 (8%) of which were greater than 100 ppm.

NES Test Performance

Univariate statistics performance on the three NES tests during the three test sessions are presented in Table 2. Midday and afternoon performance was better than early morning performance for all three tests. Test-retest correlations were high for all three tests, averaging about .8 (range .73 to .88), indicating utility of the tests for application in repeated-testing situations.

The results of exposure-response analyses are presented in Table 3. Performance on the tests was related to age and level of education, but not to years of exposure or self-report of current alcohol consumption. Performance on Symbol-Digit during the afternoon session was significantly related to styrene exposure, while adjusting for age and education. The Symbol-Digit performance was related to the natural logarithm of the personal air samples of styrene corrected for respirator use ($p=0.03$). The natural logarithm of UMA was also significantly related to Symbol-Digit performance ($p<0.03$). An additional regression model was fitted with a dichotomous variable of greater or less than 50 ppm substituted for the continuous styrene exposure variable (Table 3). These analyses showed a significant effect of this exposure variable on Symbol-Digit performance in the afternoon testing session ($p<0.02$). Within-subjects analyses (i.e., using each subject's morning performance as a covariate) accounted for much more of the total variance in afternoon Symbol-Digit performance than the cross-sectional analyses (i.e., $r^2=.69$ vs. .24) when using log mandelic acid as the exposure variable.

There was no suggestion of an effect of styrene exposure on CPT or Handeye performance. Neither were there significant effects of exposure duration on performance of any of the three tests in the morning testing session. However, these measures were not globally insensitive; they were related to age, education

TABLE 1

BASIC DEMOGRAPHIC AND EXPOSURE VARIABLES BY COMPANY

		Company						
		1	2	3	4	5	6	Total
Number	N	12	24	7	29	15	18	105
Age	Mean	41.2	29.1	29.1	28.9	33.7	41.4	33.2
(years)	Std.	14.4	9.6	5.8	8.7	9.0	7.5	10.7
Education	Mean	12.4	11.9	12.1	11.8	12.5	11.8	12.0
(years)	Std.	1.9	0.9	0.4	1.0	1.5	2.1	1.4
Alcohol	Mean	4.9	7.7	2.9	14.8	5.1	12.8	8.8
(No. drinks/week)	Std.	4.7	9.4	2.3	11.4	5.4	12.5	9.9
Exposure	Mean	9.4	1.9	1.9	1.9	6.6	8.9	4.6
Duration (years)	Std.	10.7	3.4	3.3	2.0	7.4	8.1	6.7
Styrene	Mean*	36.6	6.0	3.7	20.1	13.5	16.4	13.5
(ppm)	Std.*	5.5	5.0	5.5	2.7	6.0	4.1	5.0
Mandelic Acid	Mean*	221.4	99.5	14.9	330.3	109.9	330.3	164.0
(mg/g creatinine)	Std.*	4.1	5.5	11.0	2.7	3.0	2.7	5.0

*Geometric Mean and Std.

and time of day. In this sample neither styrene in breathing air nor UMA were related to age or education, which are the major effect modifiers of performance on the NES tests (r's<.16). Exposure duration was correlated moderately with age (r = .59) and weakly with UMA (r = .22) and with acute styrene exposure (r = .22). The magnitude of these correlations indicates that colinearity among these variables was not likely to have introduced bias in the regression analyses performed.

Additional analyses were performed similar to the regression analyses reported, but with a dichotomous variable of greater or less than 50 ppm substituted for the continuous styrene exposure variable. These analyses showed a significant effect of this exposure variable on Symbol-Digit performance in the afternoon testing session ($p<0.02$).

DISCUSSION

Exposure to levels of styrene found in this sample of fiberglass boatbuilding companies was related to some disruptions of neurobehavioral performance. Exposure to greater than 50 ppm for an 8-hour TWA appeared to account for the bulk of this effect. These results are generally consistent with previous studies of styrene exposure (3, 6, 7, 10). There is one exception (12), in which neurobehavioral performance deficits are reported among workers with styrene exposure less than 50 ppm. There were many differences between that study and the present one, the most notable being that the workers had been exposed for a mean of 8.6 years, and there was a significant relationship between exposure duration and neurobehavioral performance. In addition, styrene exposure was estimated from UMA collected the morning after exposure. In the present study the mean duration of exposure was 4.6 years, and UMA was determined from spot urine samples at the end of the work shift.

This sample of companies was not random, but the exposures were probably not atypical of many in the United States. If anything, styrene exposures in the participating companies were

TABLE 2

COMPARISON OF NES TEST PERFORMANCE BETWEEN TEST SESSIONS

				Difference From A		Correlation	
Variable	N	Mean	Std.Dev.	Mean	Std.Dev.	With A	With B
CPT							
Morning (A)	105	395.7	43.8	—	—	—	—
Midday (B)	100	383.2	40.5	12.7	25.8	.812	—
Afternoon (C)	103	384.8	41.2	10.9	31.3	.731	.827
Handeye							
Morning (A)	104	1.58	0.32	—	—	—	—
Midday (B)	101	1.50	0.28	0.09	0.19	.813	—
Afternoon (C)	104	1.48	0.28	0.11	0.22	.743	.878
Symbol-Digit							
Morning (A)	105	2.53	0.65	—	—	—	—
Midday (B)	100	2.35	0.47	0.19	0.39	.796	—
Afternoon (C)	104	2.41	0.62	0.11	0.37	.826	.860

TABLE 3

RESULTS OF CROSS-SECTIONAL EXPOSURE-RESPONSE REGRESSION ANALYSIS

Acute Exposure Variable in Model	Regression Coefficient (probability parameter = 0) Acute Exposure	Age	Education	Exposure Duration	R-square
ppm Styrene	.003 (.002)	.017 (.006)	−.121 (.004)	.007 (.667)	.230
ppm Styrene* (corrected for respirator use)	.080 (.030)	.019 (.006)	−.118 (.004)	.004 (.684)	.235
Mandelic Acid* (mg/g creatinine)	.037 (.024)	.019 (.005)	−.113 (.006)	.004 (.730)	.238
>50 ppm Styrene (dichotomous)	.350 (.013)	.016 (.014)	−.127 (.002)	.005 (.621)	.247

*Natural logarithm of this variable was used.

probably slightly lower on average than in the industry as a whole. Management at these companies agreed to participate because they did not believe that they had exposure problems. In addition, employees who had worked at other companies reported that previous exposures seemed higher than those at their current jobs. Since the primary hypotheses of the study involved acute exposure to styrene and the comparisons to be made were within subjects, random selection of companies and participants as well as having exposures representative of the boatbuilding population were not considered essential.

NES is useful for application in field investigations of exposure to potential neurotoxicants in the workplace. The three tests used in the current study exhibited high test-retest correlations, as well as measuring expected effects such as age, education, and time of day. Acceptance of the computerized testing protocol by the blue-collar participants, essential for successful completion of field studies such as this was excellent. Thus, NES can be expected to work well in other field testing situations where quantification of subclinical neurobehavioral deficits is desired.

ACKNOWLEDGEMENTS

This project was supported by a grant from the U.S. National Institute for Occupational Safety and Health (7 R01-OH002384-01). Marilyn Hallock was instrumental in collection of industrial hygiene data, and Dr. Katherine Hammond performed laboratory analysis of samples. The assistance of the management of the boatbuilding companies participating in this study, Dr. Frank Mirer and Local 1251 of the United Auto Workers, and, most importantly, the study participants is gratefully acknowledged.

REFERENCES

1. Baker, E. L.; Letz, R.; Fidler, A. T. A computer-based neurobehavioral evaluation system for occupational and environmental epidemiology: Rationale, methodology and pilot study results. J. Occup. Med. 27:206–212; 1985.
2. Baker, E. L.; Letz, R.; Eisen, E. A.; Pothier, L. J.; Plantamura, D. L.; Larson, M.; Wolford, R. Neurobehavioral effects of solvents in construction painters. J. Occup. Med. 30:116–123; 1988.
3. Cherry, N.; Waldron, H. A.; Wells, G. G.; Wilkinson, R. T.; Wilson, H. K.; Jones, S. An investigation of the acute behavioral effects of styrene on factory workers. Br. J. Ind. Med. 37:234–240; 1980.
4. Eisen, E. A.; Letz, R.; Wegman, D. H.; Baker, E. L.; Pothier, L. Defining measurement precision for effort-dependent tests: The case of three neurobehavioral tests. Int. J. Epidemiol. 17:292–298; 1988.
5. Fidler, A. T.; Baker, E. L.; Letz, R. The neurobehavioral effects of occupational exposure to organic solvents among construction painters. Br. J. Ind. Med. 44:292–308; 1987.
6. Gamberale, F.; Lisper, H. O.; Olsen, B. A. The effect of styrene vapour on reaction time of workers in the plastic boat industry. In: Horvath, M., ed. Adverse effects of environmental chemicals and psychotropic drugs. Amsterdam: Elsevier; 1976:135–148.
7. Götell, P.; Axelson, O.; Lindelöf, B. Field studies on human styrene exposure. Work Environ. Health 9:76–83; 1972.
8. Greenberg, B. D.; Moore, P. A.; Letz, R.; Baker, E. L. Computerized assessment of human neurotoxicity: Sensitivity to nitrous oxide exposure. Clin. Pharmacol. Ther. 38:656–660; 1985.
9. Hooisma, J.; Platella, S.; Twisk, D. A. M.; Muijser, H. Experimental exposure to alcohol as a model for the evaluation of neurobehavioral tests. Toxicology 49:459–467; 1988.
10. Lindström, K.; Härkönen, H.; Hernberg, S. Disturbances in psychological functions of workers occupationally exposed to styrene. Scand. J. Work Environ. Health 3:129–139; 1976.
11. Mahoney, F. C.; Moore, P. A.; Baker, E. L.; Letz, R. Experimental nitrous oxide exposure as a model system for evaluating neurobehavioral tests. Toxicology 49:449–457; 1988.
12. Mutti, A.; Mazzucchi, A.; Rustichelli, P.; Frigeri, G.; Arfini, G.; Franchini, I. Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsychological functions. Am. J. Ind. Med. 5:275–286; 1984.
13. National Institute for Occupational Safety and Health. Criteria for a recommended standard: Occupational exposure to styrene. U.S. Department of Health and Human Services Publication No. 83–119; 1983.
14. Ogata, S.; Sugihara, R. High performance liquid chromatographic prodecure for quantitative determination of urinary phenylglyoxylic, mandelic and hippuric acids as indices of styrene exposure. Int. Arch. Occup. Environ. Health 42:11–19; 1978.