Effects of Mixed Organic Solvents on Neuromotor Functions among Workers in Buddhist Altar Manufacturing Factories

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Department of Environmental Health Sciences, Akita University School of Medicine—To clarify the neuromotor effects of long-term exposure to mixed organic solvents, postural sway and tremor were measured in 62 solvent workers of four Buddhist altar manufacturing factories who had worked for 1–46 (mean 12) yr. By using the passive gas sampler, 8-h time-weighted average concentrations in the workers were estimated to be 0.02–8.7 ppm for toluene, 0.02–7.7 ppm for xylene, 0.02–5.5 ppm for styrene and 0.02–40.5 ppm for n-hexane. Sagittal sway and sway area of the posturography with eyes closed were significantly larger in the solvent workers than in 35 age-matched controls (p<0.05), and there was a significant difference in Romberg quotient of sagittal sway between the two groups (p<0.05). Also, tremor intensities at 1.0–5.9 Hz, 6.0–9.9 Hz and 10.0–13.9 Hz with the right hand, and at 6.0–9.9 Hz with the left hand were significantly stronger in the solvent workers than in the controls. Among the solvent workers, transversal and sagittal sways with eyes open and tremor intensity at 10.0–13.9 Hz were significantly related to toluene exposure (p<0.05), which may have been due to the acute effects of such solvents. These findings suggest that long-term exposure to mixed organic solvents may impair neuromotor functions as measured by postural sway and tremor.

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Little information has been developed regarding hand tremor in workers exposed to organic solvents, although styrene, n-hexane, and benzene have been reported to increase postural sway1–4. Since a toluene-induced tremor has been demonstrated in a 48-yr-old worker occupationally exposed to the substance5 and in a 22-yr-old man with a 7-yr history of chronic toluene misuse6, such tremors may be objectively detected even in workers exposed to subclinical levels of organic solvents if a specific test exists.

There are about 180 small-scale factories that manufacture Buddhist altars in a rural area of the Tohoku district (the northeast part of Japan). Most of these were established in the early 1960s, and most are “cottage industries”—literally located inside the owner’s residence. The manufacturing process consists of grinding, gilding, painting, and assembling, and various solvents (for example, styrene in grinding with putty, toluene and xylene in painting, and n-hexane and white gasoline in gilding with Japanese lacquer) are used. The organic solvents differ according to each home factory. In addition, since workers have moved about in the small factories, they have been exposed to a mixture of different organic solvents4, 8–10. In each process, a certain solvent concentration in the air may have been particularly high because such work (e.g., grinding, gilding or painting) is carried out in a windtight room to avoid airflow. Nevertheless, few detailed surveys on the adverse health effects of solvent exposure have been conducted in these places for a couple of decades, though improvement of facilities such as local exhaust or general ventilation may have been made to some extent at each factory. In this study, postural sway and tremor were measured in workers of four Buddhist altar manufacturing factories that cooperated, to clarify the neuromotor effects of long-term exposure to mixed organic solvents.

Subjects and Methods

Subjects

Thirty-five men and 29 women from the above
factories were examined at the time of the specific health examination for organic solvent workers, conducted under the Industrial Safety and Health Law in Japan. Two men were excluded from the study population because of a lack of exposure data. The remaining 62 workers had been engaged in grinding, assembling, painting, or gilding in the cottage industries, and had worked in a similar manner for 1–46 (mean 12) yr. Geometric mean concentrations of chemicals in the air in four factories were 19.3 (range 1–98.5) ppm of styrene in the grinding workroom, 6.4 (3.6–20.2) ppm of toluene and 4.6 (1.3–27.5) ppm of xylene in the painting workroom, and 9.4 (3.5–20.4) ppm of n-hexane in the gilding workroom on the day of the examination. The age-matched control group consisted of 14 male and 21 female healthy volunteers residing in the area near the above factories. They were all doctors, nurses or office workers who had not been exposed occupationally to neurotoxic chemicals such as organic solvents, manganese (Mn), mercury (Hg), or lead. All of the solvent workers and control subjects were right-handed. The nature of the procedure used in the present study was fully explained to all subjects, and the study was carried out with their informed consent.

Methods
Questions about job content, health condition, and smoking and drinking habits were asked by an occupational practitioner (IO). A total of 100% ethanol equivalent dose (g/wk) was calculated for each subject, using a questionnaire reported previously[9]. In the solvent workers, concentrations of toluene, xylene, styrene, and n-hexane were measured using passive gas samplers (Sibata Co., Japan) worn through the day, and were estimated as 8-h time-weighted average concentration (ppm) by gas chromatograph mass spectrometry (Q-mass910 Mass Spectrometer; Perkin Elmer Inc., USA) with Spelcowax 10 column separation and single ion monitoring detection.

Neuromotor function measurements for each subject were conducted by one examiner (TI) at each factory for 10 min during work hours (i.e., at 8:30–17:30) according to the methods described by Després et al.,[12] using a neuromotor test battery (CATSYS 2000; Danish Product Development Ltd., Denmark). Regarding the postural sway, subjects were asked to stand up straight on a platform, feet 1 cm apart with their arms at their sides[13]. The frequency analysis of tremor intensity was conducted with the fast Fourier transform method. These functions did not seem to be affected by a diurnal variation because no special conditions such as sleep deprivation or night work existed[14–16]. Also, postural sway in children tends to be larger in boys than in girls, but tremor does not differ according to sex[17].

The comparison between the exposed and unexposed subjects was performed by the analysis of covariance with covariates such as age, gender, smoking status, and alcohol consumption (and height, when the postural parameters were analyzed). Smoking status was scored as “non-smoker or ex-smoker”=0 and “smoker”=1. The relations of toluene, xylene, styrene, n-hexane, and possible confounders with each neuromotor parameter were tested by the following multiple regression model[4, 9, 10]:

\[
\text{[neuromotor parameter]} = b_0 + b_1 \cdot \text{[toluene]} + b_2 \cdot \text{[xylene]} + b_3 \cdot \text{[styrene]} + b_4 \cdot \text{[n-hexane]} + b_5 \cdot \text{[age]} + b_6 \cdot \text{[gender]} + b_7 \cdot \text{[smoking status]} + b_8 \cdot \text{[alcohol consumption]} + b_9 \cdot \text{[height]}.
\]

Also, the relationship between postural sway and tremor parameters was analyzed by canonical correlation analysis. All analyses, with two-sided p values, were performed using the Statistical Package for the Biosciences (SPBS V9.51)[10].

Results
The basal characteristics and solvent exposure levels of male and female workers are shown in Table 1. There were no significant differences in age, height, alcohol consumption or smoking status between the solvent workers and control subjects (Mann-Whitney U test or Fisher exact probability test, p>0.05). None of the workers reported any neurological or motor disturbances other than presbyopia and lumbago. Sagittal sway and sway area with eyes closed were significantly larger in the 62 solvent workers than in the 35 control subjects (Table 2); also, there was a significant difference in Romberg quotients of sagittal sway between the two groups. Tremor intensities at 1.0–5.9 Hz, 6.0–9.9 Hz and 10.0–13.9 Hz with the right hand, and at 6.0–9.9 Hz with the left hand were significantly stronger in the solvent workers than in the control subjects (Table 2). However, no significant relationship was found between the neuromotor parameters and working duration (yr) after controlling for possible confounders (p>0.05, data not shown).

As shown in Table 3, transversal and sagittal sways of the posturography with eyes open were significantly related to toluene exposure in the solvent workers, and a negative relationship between the transversal sway with eyes closed and styrene exposure was found. The Romberg quotients were not associated with any organic solvents in the workers (p>0.05, data not shown). Tremor
intensity at 10.0–13.9 Hz was significantly related to toluene exposure in the 62 workers. On the other hand, alcohol consumption was not significantly related to any neuromotor functions in the regression analysis.

The canonical correlation coefficients between postural sway parameters (transversal sways, sagittal sways and sway areas with eyes open/closed) and tremor parameters (tremor intensities at 1.0–5.9 Hz, 6.0–9.9 Hz and 10.0–13.9 Hz with right/left hands) were 0.853 ($p=0.0003$) for the 35 control subjects and 0.715 ($p=0.0002$) for the 62 solvent workers. Especially, the tremor intensity at 1.0–5.9 Hz with the right hand was significantly correlated...
with all postural sway parameters in the control subjects
(0.334 ≤ simple correlation coefficient \( r \) ≤ 0.605, \( p < 0.05 \))
and with all postural sway parameters in the solvent
workers except sagittal sway (0.270 ≤ \( r \) ≤ 0.491, \( p < 0.05 \)).
Figure 1 illustrates the relationship between sagittal sway
with eyes closed and tremor intensity with the right hand.

**Discussion**

In the solvent workers who had a mean employment
time of 12 yr at Buddhist altar manufacturing factories,
ppm for n-hexane\textsuperscript{(9)}. However, concentrations may have been considerably higher in the past, because some new local exhaust and general ventilation systems have been set up at the factories, and one of those factories, in fact, was recommended to install such ventilation systems by the local labor inspector 15 yr ago. These findings agree with previous reports on workers exposed to a different mixture of organic solvents, \textit{e.g.}, industrial solvents (mainly, styrene)\textsuperscript{1, 2}, jet fuel (benzene, toluene and xylene)\textsuperscript{3}, and n-hexane, xylene and toluene\textsuperscript{4}. In addition, Yokoyama \textit{et al}. have postulated vestibulo-cerebellar effects of n-hexane, based on significantly increased sagittal sway at 2–4 Hz and transversal sway with eyes open in the solvent workers\textsuperscript{4, 20}. In the light of the previous literature, therefore, long-term exposure to mixed organic solvents appears to affect postural balance.

The dominant hand (\textit{i.e.}, right hand) in the solvent workers of our study also showed more tremor when compared with that in the control subjects. As mentioned in the introduction, toluene-induced tremor has been observed only in case reports\textsuperscript{5-6, 21}, and there has been no epidemiological study on the effect on tremor of exposure to a subclinical level of organic solvents except ethanol\textsuperscript{22}. Apart from the risk assessment of organic solvents, Mn-exposed workers with a geometric mean Mn level of 181 nmol/l in whole blood had increased hand tremor compared with the age-matched referents with that of 160 nmol/l\textsuperscript{(22)}, probably indicating that the tremor test would be sensitive to occupational hazardous substances. For that reason, this report may be the first to suggest that long-term exposure to mixed organic solvents induces asymptomatic tremor.

This investigation involved four factories. Since solvents used and workroom conditions differed according to each factory\textsuperscript{7}, we evaluated personal exposure levels by using a passive gas sampler. When two or more organic solvents acting upon the nervous system are present, their combined effect, rather than that of either individually, should be given primary consideration\textsuperscript{(9)}. For this reason, we employed four solvent concentrations, together with confounders such as age, gender, smoking status, and alcohol consumption, as independent variables in the multiple regression model, as other researchers did previously\textsuperscript{4, 9, 10}. In the mixture of organic solvent exposure (Table 3), toluene was suggested to be the principal risk factor in neuromotor impairment, and styrene might have a protective effect on sagittal sway with eyes closed. However, these findings may imply not “chronic” but “acute” effects of such solvents because the biological half-life of these chemicals is considerably shorter than that of metals such as lead and cadmium\textsuperscript{23}. Yokoyama \textit{et al}. have suggested the possibility that xylene can inhibit the effect of n-hexane on postural balance, based on a similar regression model\textsuperscript{4}. Such interactions of mixed organic solvents on the nervous system have not been clearly elucidated and may depend on the configuration and size of neurons, although styrene is considered to be a neurotoxic substance even at considerably low exposure levels\textsuperscript{2, 25, 26}. Additional research is required to clarify the interactive effects of styrene on neuromotor function, as well as to identify the most appropriate indicator of exposure monitoring for each organic solvent.

Alcohol consumption in the solvent workers had no relation to any neuromotor parameters (Table 3). This seems to be concordant with the finding that acute exposure to ethanol at levels of less than 1,000 ppm induced no significant neuromotor alterations\textsuperscript{22}. The presence/absence of alcohol effects on neuromotor function awaits epidemiological research with a large number of subjects. On the other hand, our result of the multiple regression analysis indicated that smoking status was associated with the neuromotor function (Table 3). This finding is consistent with two reports published previously: that is, smokers exhibited more unstable posturographic results than non-smokers\textsuperscript{27}, and Mn-exposed smokers had more hand-tremor than Mn-exposed non-smokers in Mn-alloy-producing plants\textsuperscript{23}. Thus, smoking habit is one of the important confounders in assessing postural sway and hand tremor.

In the present study, some positive associations between postural sway and tremor were observed both in the unexposed subjects and in the exposed workers. Specifically, the tremor intensity at 1.0–5.9 Hz with the right hand had close relation to postural sway parameters (Fig. 1), implying that the tremor in the low-frequency band may have resulted from postural sway because the upper body of the examinee was freed from the back of a chair; whereas, the largest power of postural sway appeared to lie in the frequency band of 0–1 Hz in healthy men\textsuperscript{8}. The reason why the relation of tremor with postural sway differed between the right and left hands may have been attributable to the fact that all the subjects of our study were right-handed. Therefore, tremor in the high-frequency band (\textit{i.e.}, at 6.0–9.9 Hz), in which a significant difference was found between the solvent workers and controls in this study, may have been due to specific changes to mixed organic solvents. Further research is necessary to explain the mechanism of tremor involved in organic solvents. Also, since some hazardous substances such as Hg and Mn have been described as inducing tremor\textsuperscript{23, 24}, tremor tests may provide quantitatively useful information on neuromotor functions in health risk evaluations.

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