Audiological findings in workers exposed to styrene alone or in concert with noise

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ABSTRACT

Audiological testing, interviews and exposure measurements were used to collect data on the health effects of styrene exposures in 313 workers from fiberglass and metal-product manufacturing plants and a mail terminal. The audiological test battery included pure-tone audiometry, distortion product otoacoustic emissions (DPOAE), psychoacoustic modulation transfer function, interrupted speech, speech recognition in noise and cortical response audiometry (CRA).

Workers exposed to noise and styrene had significantly poorer pure-tone thresholds in the high-frequency range (3 to 8 kHz) than the controls, noise-exposed workers and those listed in a Swedish age-specific database. Even though abnormalities were noted on DPOAE and CRA testing, the interrupted speech and speech recognition in noise tests were the more sensitive tests for styrene effects. Further research is needed on the underlying mechanisms to understand the effects of styrene and on audiological test batteries to detect changes in populations exposed to solvents.

Keywords: Cortical response audiometry, hearing loss, noise, otoacoustic emissions, sensitized speech tests, solvents

Introduction

Styrene exposure causes permanent and progressive damage to the auditory system of the rat and several experiments have revealed that noise interacts with styrene[1-3] in a synergistic manner. This combination of effects poses serious implications because noise is likely to occur in settings where there are occupational styrene exposures. The rat experiments showed that age also plays a role in the interaction between noise and styrene, as young rats have significantly less outer hair cell loss than older animals.[3] In addition, styrene has been shown to be a more potent ototoxicant than toluene in rats.[4]

Several field studies have been conducted with styrene-exposed workers in boat or fiberglass products manufacturing. Muijser *et al*[5] reported that workers exposed to low levels of styrene did not appear to have increased hearing loss at high frequencies when compared to controls. The comparison of the two extreme exposure groups (low and high exposures), however, revealed a statistically significant difference in hearing thresholds at high frequencies.

Styrene and noise exposures were assessed for 299 workers in the fiberglass industry.[6] Noise levels ranged between 85 and 90 dB(A), while styrene levels were generally below the Canadian recommended limit of 50 parts per million (ppm). The association between noise exposure (based on the developed lifetime noise dose estimate) and hearing loss (assessed by averaging pure tone thresholds) was significant. No such association was found between hearing loss and styrene exposure. Hearing loss approached significance for styrene exposure only at some specific frequencies, 4 and 6 kHz, in the left ear.[6]

The effects of styrene were also investigated in male Japanese workers exposed in factories that produced plastic buttons or bathtubs.[7,8] Medical examinations, audiological evaluations and exposure assessments to noise and solvents (in air and

urine) were conducted in both investigations. In the 1999 study, workers whose noise exposures exceeded 85 dBA were excluded from the study population. Participants were exposed to a mixture of solvents containing mainly styrene and toluene. Of the 93 participants, only 6 were exposed to levels of styrene that exceeded the Japanese exposure limit (50 ppm) and 2 were exposed to toluene levels exceeding a similar limit of 50 ppm.[7] In the 2000 study, 48 study participants were divided into three subgroups by their exposure condition: an unexposed group, a group exposed to low levels of styrene (2.9 to 28.9 ppm) and noise (69 to 76 dBA) and a group exposed to noise levels that ranged from 82 to 86 dBA.[8] No effects of the solvents were detected by conventional pure-tone audiometric testing, but the detection of high frequency tones was reduced in workers exposed to styrene for five years or more. This effect was associated with styrene concentrations in air and mandelic acid concentrations in urine.

As part of a large investigation conducted in Poland,[9] a group of styrene-exposed workers (n=194) was compared with groups of workers exposed to styrene and noise (n= 56), styrene and toluene (n=26), styrene, toluene and noise (n=14), noise alone (66) and unexposed (n=157). The study protocol included a questionnaire; assessments of styrene, other solvents and noise exposures; and an audiologic test battery. The questionnaire, an adapted and translated version of the protocol used by Morata *et al*[10] included questions on work history, non-occupational solvent and noise exposure and medical history. The participants from the styrene group were exposed to noise levels ranging from 78 to 86 dBA. Styrene average exposures (8 h values) in the styrene groups ranged from 11 to 38 ppm and had a maximum exposure of 120 ppm. Hearing loss was observed in 76% of the workers exposed to styrene and noise or styrene and toluene, in 57% of the styrene-only group, 56% in the noise-alone group and 33% in the unexposed group. Significantly higher mean audiometric thresholds ($P<0.05$) were observed in the styrene-exposed workers at 2, 4 and 6 kHz when compared to the noise-only and the unexposed groups. When compared to the styrene- and toluene-exposed groups, mean thresholds were also significantly higher at the frequencies of 4 and 8 kHz. The odds ratio estimates for hearing loss were also the highest among all groups exposed to styrene.

In a clinical study, 18 workers underwent pure-tone audiometry and a diagnostic audiological and otoneurological test battery.[11] Routine audiometric results of workers exposed to styrene in the plastic boat industry did not indicate hearing losses resulting from causes other than exposure to noise.[11] Seven of eighteen workers, however, displayed abnormal results in the distorted speech tests and cortical response audiometry, as well as in some of the otoneurological tests performed.

In 2002, Morata *et al*[10] reported the results of the initial analysis performed on pure-tone audiometry data, part of a larger dataset being reported in the present paper. The initial analysis indicated an association of the biological determinant of styrene and auditory dysfunction. About 60% of the participants in both groups exposed to noise (styrene and noise and noise alone) were exposed to levels above the Swedish limit value (85 dBA/3 dB exchange rate) and the range of exposures was also similar in these groups (75-116 dBA). Styrene exposures were low, averaging 3.5 ppm and having a maximum value of 22 ppm over 8 hours (h). The 8 hour occupational exposure limit in Sweden is 20 ppm. Workers exposed to noise and styrene had significantly worse pure-tone thresholds at 2, 3, 4 and 6 kHz when compared with noise-exposed or non exposed workers. From the numerous variables that were analyzed for their contribution to the development of hearing loss, the only ones that met the significance level criterion in the final multiple logistic regression model were age, noise exposure (past and current) and urinary mandelic acid levels (one of the biologic markers for styrene). The odds ratio estimate for hearing loss was 2.44 times greater for each mmol of mandelic acid per gram of creatinine (95% CI: 1.01-5.88), 1.18 times greater for each dB of current noise exposure (cumulative exposure index, 95% CI: 1.01-1.38) and 1.19 greater for each year of age (95% CI: 1.11-1.28).

The objective of the present cross-sectional study was to re-examine data from Swedish workers exposed to styrene alone or in combination with noise, using a questionnaire, assessment of styrene and noise exposures and an extensive audiologic battery. This paper gives the results of the audiological test-battery, including pure-tone audiometry, distortion product otoacoustic emissions (DPOAES), psycho-acoustical modulation transfer function (PMTF), distorted speech, speech recognition in noise and cortical response audiometry.

Materials and Methods

Workers exposed to styrene alone or in combination with noise were evaluated in Sweden, using a questionnaire, assessment of styrene and noise exposures and an extensive audiologic battery. The questionnaire gathered information on work history, non-occupational solvent and noise exposure, life style factors and medical history. Exposure assessments included data collected from interviews, company records and site measurements of noise levels for different work tasks. Styrene measurements were conducted on all exposed workers by air samples and biological monitoring of mandelic acid in urine.

Participants

The fiberglass products (FGP) industry is one of the few occupational settings which has an almost mono exposure to styrene, although small amounts of acetone are also used for cleaning tools. Eleven FGP manufacturers agreed

to participate in this study, varying in size from 5 to 500 employees. Of those, 154 styrene-exposed workers participated, 65 of whom were not exposed to excessive noise levels (above 85 dBA time-weighted average). Noise-exposed controls (n=78) were selected from three companies in the metal products manufacturing industry and the non-exposed controls (n=81) were selected from a mail distribution terminal. Originally, 329 workers met the eligibility criteria and were invited to participate in the study, but 10 styrene-exposed workers did not participate - 3 due to illness at the time of hearing measurements and 7 for other undisclosed reasons. Out of the remaining 319 workers who participated in the audiometric tests, 3 were excluded from the analyses due to their inability in performing the tests and 3 more due to missing questionnaires, which caused a lack of background and exposure data. Thus, a total of 313 subjects, 278 male and 35 female (11%), were included in the study.

All styrene-exposed workers employed for a minimum of one year in the FGP companies were invited to participate in the study. The workers from the metal industry were selected based on noise exposures equivalent to those of the FGP workers. The unexposed controls were randomly chosen from a large number of employees in a mail distribution terminal. The noise levels in the terminal were below 85 dBA time-weighted average (TWA). One FGP plant was revisited four years after the end of the initial data collection and 30 of the workers were reexamined using the same exposure measurements and questionnaire as before, but with a new pure-tone audiometry.

The project was approved by the Ethical Committee of the Stockholm region at Karolinska Institutet.

Styrene exposure assessment

Since details of the exposure assessment and the analyses of the samples were described in an earlier publication,[10] they will just be summarized here. To determine the level of exposures to styrene, TWA exposure evaluations were conducted on all subjects exposed to styrene and on five subjects from each of the other groups for control purposes only. Passive samplers were used and two successive samples were collected for each worker. The adsorption tube samples were then sealed and stored in a freezer for later gas chromatography (GC) analysis.

Total styrene exposure was assessed also by the biological monitoring of mandelic acid and creatinine in the urine collected over 24 hours, beginning with the start of the work shift. Samples of urine were then taken for analyses by high-pressure liquid chromatography (LC).

Noise exposure assessment

Details of the noise exposure assessment were described earlier[10] and will only be summarized here. Noise exposure was assessed by personal exposure measurements using noise dosimeters [Brüel and Kjær 4436]. Exposure assessments were calculated individually, based on eight-hour level equivalent dosimeter measurements. At least, one full-shift noise dosimetry was performed for all different work tasks. For the workers (n = 128) whose personal noise dosimetry was not performed, a mean value of the noise levels obtained from workers doing the same work tasks was used in the analyses.

Estimates of workplace exposures from previous jobs were calculated based on questionnaire information. To illustrate, workers were asked if their previous workplace had been "quiet" or "very noisy." Information on typical noise levels in certain industries was found in the literature[12] and included in the estimation of past exposure. For each subject, the total cumulative noise and styrene exposures were calculated, using the records of the mandatory exposure measurements made by each company, at least, over a 10-year span. For details, see Morata *et al.*[10] The styrene lifetime exposure values reported for the noise only groups refer to past exposures to styrene during brief periods of time. The characteristics of the study population groups (control, noise only, styrene exposed and styrene and noise) regarding their age, tenure, current and previous exposures to the studied agents are presented in Table 1.

Table 1: Characterization of the study population (n=313). Mean values and range (within parenthesis) for the variables of age, tenure, previous noise exposure, current noise and styrene exposures and estimated lifetime noise and styrene exposures

	Control (n=78)	Noise (n=65)	Styrene (n= 89)	Styrene and noise (n=81)
Age (years)	45 (26-62)	42 (20-64)	43 (21-62)	43 (21-65)
Tenure* (years)	18 (2-38)	12 (1-35)	17 (1-39)	15 (2-37)
Previous noise exposure (≥ 85 dBA TWA) (years)	7 (0-25)	12 (1-26)	5 (0-16)	6 (0-21)
Exposures				
Current noise level (dBA)	77 (69-86)	85 (75-116)	82 (75-84)	89 (85-108)
Lifetime noise exposure (dBA)	79	86	84	89
Current styrene (mg/m³)	0a	0a	16 (0.2-96)	12 (0.03-50)
Mandelic acid in urine (mmol/g creatinine)	0a	0a	0.9 (<MDC-2.9)	0.9 (<MDC-3.0)
Lifetime styrene (mg-years/m³)	0	22.04	1303	884

*Variables that met the significance level criterion ($P<0.0001$) for differences between groups, [a]Random samples taken in groups, no levels detected,
MDC - Minimum detectable concentration.

Testing the auditory system

A mobile laboratory, a converted bus, had two soundproof booths installed that met the requirements of the American National Standards Institute (ANSI) S 3.1, 1991 for audiometric testing environments. Otoscopy was performed to screen for conditions that would exclude a person from the study, i.e., excessive cerumen, external otitis or perforated tympanic membrane.

The test battery was chosen to include tests of the different parts of the auditory system. Damage to the peripheral auditory system was tested with pure-tone audiometry, which is sensitive to cochlear impairments and the classic measure of the status of the hearing system as a whole. Outer hair cell function was also investigated by DPOAEs and PMTF tests, which show the ability of the ear to follow intensity modulations of various frequencies.[13] The central auditory pathways were investigated through speech discrimination tests and cortical auditory evoked responses.

Equipment

A technical audiological measurement processor (TAMP3), constructed at the unit of Technical Audiology of the Karolinska Institute and controlled by a personal computer was used to test pure-tone, speech audiometry and psycho-acoustical modulation transfer function. TAMP3 is based on a Texas TMS32010 signal processor, having a 96 kB memory, A/D- and D/A-converters, a real-time clock, anti-aliasing filters, controllable attenuators, amplifiers with controllable gain and an output amplifier suited for the headphone type TDH-39, with MX41AR cushion. Software was developed for calibration, pure tone audiometry and PMTF.

The equipment used to measure the DPOAEs was based on Tucker-Davis technologies modules and controlled by a personal computer with software developed at the unit of Technical Audiology. The system included a signal processing board, microphone probe, signal sources, earphones ER-3 and preamplifier. The microphone (Etymotic Research ER-10) incorporated the signal delivery and microphone with a soft foam ear tip into a small package that was inserted in the subject's ear canal.

Essentially the same equipment from Tucker-Davis technologies that was used for measuring DPOAE, but with the addition of preamplifier Entomed 510, was used for cortical response audiometry (CRA). A separate software program was employed for the CRA.

The speech materials were stored on the computer hard disc that was connected, through a controllable attenuator, to the output amplifier. The latter two were parts of the TAMP3 equipment. The output level 70 dB SPL was set by a computer program (calibrated in a 6cc coupler). The speech lists were presented through a TDH-39 headphone. For speech recognition in noise, an extra controllable attenuator and a mixer that added the noise to the speech signal was added to the output amplifier.

Pure-tone audiometry

Pure-tone thresholds were measured with the fixed frequency, pulse tone Békésy method for both ears at the frequencies 1 (twice), 2, 3, 4, 6 and 8 kHz. The first threshold at 1 kHz served as training and was not used for the analysis. Most of the following measurements were performed only on the best ear, chosen by comparing the mean threshold values for the two ears between 1 and 8 kHz. Mean thresholds were compared among groups as reported in Morata *et al.*[10] In the present paper, individual thresholds were compared to validated material of an otological unscreened, non-occupational noise-exposed population (n=603; age 20 - 79 years) from Sweden,[18] which became available after the initial analysis of the audiometric data in 2002.[10]

Psycho-acoustical modulation transfer function

PMTF test shows the ear's ability to follow intensity modulations, which is essential for speech recognition. The threshold of a 4 ms brief tone, centered in a fluctuating octave-filtered noise was measured. The threshold of the probe tone was measured also without noise (i.e., a brief-tone threshold). Separate thresholds of tone detection were measured at the peaks and at the valleys of the modulated noise. A signal-to-noise (S/N) ratio at threshold was used as the resulting measure. Thresholds were determined with an adaptive method (Békésy-technique). Threshold for the brief tones was measured at noise levels from 25 to 95 dB sound pressure level (SPL) in steps of 10 dB. The noise consisted of the octave-band around the test tone of 4000 Hz, with a sinusoidal 100% intensity-modulation of 10 Hz. To prevent listening to sounds outside the octave-band, a masking noise of a faint, periodic broadband noise was added. The degree of accuracy has been found to be about 2 dB for peak and valley thresholds.[13] A level dependence of the PMTF showed that the best ability (i.e., the biggest difference between the peak and the valley threshold) normally is found at noise levels in the range of normal speech levels, i.e., 55-65 dB SPL. For hearing impaired subjects the best ability occurs at higher levels.

DPOAE

For the testing of DPOAE, two continuous tones were presented to the ear at frequencies f1 and f2 while the ratio of the frequencies was maintained at f2/f1=1.225. The level of f2 was 10 dB lower than the level of f1. The tones were presented for 4.3 seconds and the frequency spectra of the responses were averaged to produce a resultant spectrum of the ear canal signal. A computer controlled the generation and sampling from the respective sources and microphone. A spectrum was calculated and the software measured the energy at the distortion product frequency 2f1-f2 and reported the DPOAE amplitude associated with the geometric mean (GM) frequency (f1 * f2)1/2 of the two primary tones. Several averages were measured until a stable value was

obtained. A response was considered to be present when the DPOAE amplitude was determined to be at least 3 dB above the background noise floor.

The input-output function of DPOAE was collected with f1 at the frequency 4 kHz for overall input levels 35 to 80 dB SPL, in steps of 5 dB.

CRA

CRA is an electrophysiologic measure that tests the central pathways of the auditory system. The stimulus used was the frequency glide of a 1000 Hz continuous pure tone at 60 dB HL. Its frequency was linearly increased 50 Hz in 20 ms, then, it stayed at 1050 Hz for 480 ms and finally returned linearly to 1000 Hz during 600 ms. The inter-stimulus interval varied randomly from 2 s and up, with a mean of 4 s. Fifty 500 ms sweeps were summed by the computer to obtain an average response. If the hearing threshold at 1000 Hz was raised to 20 dB HL or moree, the pure tone was amplified according to the "half gain rule," e.g., for a hearing threshold of 40 dB HL, the level 60 + 40/2 = 80 dB HL was used. The highest level permitted was set to 100 dB HL. Electrodes were placed with the reference electrode at the vertex of the skull and the active electrode behind the measured ear. An electrode behind the contra-lateral ear acted as ground. The signal was feed into a preamplifier and the output from the preamplifier was post processed with a linear phase digital filter with the -3 dB point at 400 Hz (and the -20 dB point at 950 Hz). The latency of the cortical response was used as the outcome measure.

Interrupted speech

IS at 7 interruptions per second was used to test the central hearing pathways. Korsan-Bengtsen[14] lists of 25 sentences, containing 100 key words each, were used. Five sentences from one separate list were presented at 70 dB SPL for familiarization with the test. If a participant asked for a higher presentation level, further sentences were presented at higher levels until a comfortable listening level was found. One test list was presented at this level. The key words correctly repeated were counted and a score was given as the test result.

A normal reference group, adapted after Korsan-Bengtsen[14] who gives the mean of 93% correct responses for older controls (mean age 55 years), was used for comparison. The results were considered below mean if the discrimination score was below 93% and abnormal when the score was below 78% (mean minus 3 standard deviations).

Speech recognition in noise

Speech recognition in noise is a test that poses great demands on the whole auditory system. It mimics a difficult listening situation often encountered in real life for a person with hearing difficulties.

An adaptive procedure was developed for measuring the speech reception threshold in noise.[15] A fixed speech level of 70 dB SPL was used. The noise level was changed, either increased or decreased a specified number of decibels after each sentence, depending on the number of correct words in a sentence. A computer program executed the level changes when the number of correct words was entered into the computer. The computer calculated the final result as the signal-to-noise-ratio (S/N) when 40% correct answers were obtained.

Three lists of Hagerman's sentences in noise were used.[16,17] One list was used as training to familiarize the subject with the test. If the subject asked for a higher presentation level than 70 dB SPL, further sentences were presented at higher intensities until a comfortable listening level was found. Finally two test lists were run. Each list consisted of 10 sentences with 5 words each, i.e., 50 words altogether. Each word was scored when incorrectly repeated.

The results were considered abnormal if the S/N exceeded -7.8 dB when compared to a normal reference group of 10 normal hearing subjects, median age 27, (range 23-30).[15]

Data analyses

To investigate the effects of styrene exposure on the auditory system, the factor "exposure group," composed of four levels-styrene and noise, styrene only, noise only and control-was tested against the dependent variables using one-way between-subjects analysis of variance (ANOVA), followed by pair-wise contrasts. The dependent variables of interest were (1) pure-tone audiometry thresholds, (2) outcome measures in the PMTF test, (3) DPOAEs, (4) CRA latency, (5) interrupted speech and (6) speech in noise data.

The pure tone audiometry thresholds were also compared to a Swedish database[18] using the following method. The proportion of persons with thresholds greater than the median (50th percentile) and 90th percentile reference values were calculated. Separate calculations were performed for best and worst ears at each frequency for each of the exposed groups; *t*-tests were carried out to determine if the proportions were significantly different from 0.50 for the median and 0.10 for the 90th percentile.

The strength of the relationship between questionnaire and exposure variables and the dependent variables was investigated using Pearson bivariate correlation, followed by the more specific analyses of variance.

The data were analyzed using the statistical analysis system (pre-production version 9.00, SAS®, SAS Institute Inc., Cary, NC) and the SPSS statistical system (version 11.5, SPSS Inc Chicago, IL.

Results

Questionnaire data

One way ANOVAs compared the means of the response scores on the medical history, occupational and non-occupational exposures, lifestyle factors, present health and tests for differences among the four groups. The questions included data on smoking, diabetes, prior ear surgery, head injury, high fever, measles, high blood pressure, mumps, ear infections, history of hearing loss in the family, ototoxic medication use and tinnitus. The only variable where the groups differed statistically was tenure (P<0.001, Table 1) and the noise-exposed workers had the shortest tenure.

Exposure assessment

Noise exposures exceeded recommended limits for 130 of the 313 studied workers. Styrene exposures never did exceed the Swedish recommended limits, which are among the world's lowest (90 mg/m^3 or 20 ppm).

Audiometry

In all four exposure groups, mean thresholds at frequencies were calculated for each ear. Results are shown in Figure 1. Significantly higher thresholds at 2, 3, 4 and 6 kHz were observed in the styrene-exposed workers in both ears, compared with the other groups, including the control.

Age-related comparison with the 10th, the median and the 90th percentiles of the Swedish database[18] are shown in Figures 2 and 3 for 4000 and 6000 Hz, respectively.

The comparison of proportions of persons in each exposed group differing from the median showed significantly greater proportions than expected (poorer thresholds) in both the styrene-exposed groups for the worst ear at 4000 Hz (P< 0.001) and 8000 Hz (P< 0.01) and for both ears at 6000 Hz (P< 0.001). In the noise group significantly greater proportions of increased thresholds relative to the median were observed for the worst ear at 6000 Hz (P< 0.001) and for both ears at 8000 Hz (P< 0.05), Table 2. Comparison with the 90th percentile showed significantly greater proportions for the styrene group for the worst ear at 6000 Hz (P< 0.05) and for the styrene and noise group and the noise group for the worst ear at 8000 Hz (P< 0.05).

Retesting of 30 workers

Styrene exposed workers who participated in the initial data collection were retested after 3 years, using pure-tone audiometry. Their noise and styrene exposures were also re-evaluated. The test results were compared with their earlier results. Neither the styrene level nor the noise measurement results showed remarkable differences over the 4-year interval. Noise levels showed a mean of 84 dB(A) (range 78-92), styrene levels were measured to a mean of 13.7 mg/m^3 (range 8.4-64.5 mg/m^3). Six (20%) of the 30 workers retested after 3 years showed a significant (greater than 10 dB HL) worsening of their auditory thresholds in at least one test frequency. In only one case did a worker show similar worsening in more than one test frequency.

PMTF

There was a difference in hearing threshold, measured with a brief tone at 4 kHz, between the styrene and noise group and the controls and between the styrene and noise group and the noise group. In the comparisons among the groups on the threshold in modulated noise, a significant difference was seen at the top value of the peak threshold curve where the noise group had a lower threshold compared to controls. The noise group, as well as the styrene and noise group contained several subjects without readable top values at the peak and valley threshold curves. Those subjects lacked the nonlinearity necessary for a reading. Statistically significant differences existed between noise (alone or with styrene) and non-noise exposed (with or without styrene) groups at P<0.05. Means and standard deviations are presented in Table 3.

DPOAE

A one-within, one-between ANOVA with factors exposure group and signal level (35-70 dB in 5-dB steps) was performed on DPOAE scores. As expected, the main effect of signal level was highly significant (F* [2.02, 34.32] = 81.34, P<0.001; MSE = 48.02).1 While the main effect of the exposure groups failed to reach significance, the interaction between exposure groups and signal level was significant (F [21, 119] = 2.12, P=0.006; MSE = 13.85; Figure 4).

Figure 4 shows that for low signal levels (up to 50 dB), control and noise groups had somewhat higher DPOAE levels compared to the two groups exposed to styrene. However, past 50 dB, the control group levelled off in contrast to all

Table 2: Proportion (%) of each group with hearing levels (dB HL) above the median level of age-correlated hearing levels from a database of a normal population in Sweden

Group	3000 Hz		4000 Hz		6000 Hz		8000 Hz	
	Best ear %	Worst ear %	Best ear %	Worst ear%	Best ear %	Worst ear %	Best ear %	Worst ear %
Control	none	none	none	4.6%	14.0% *	18.4%**	none	14.0%**
Noise	none	none	none	3.8%	1.3%	24.0%***	11.8%*	15.3%**
Styrene (N<85 dB)	none	8.5%	none	17.7%*	35.6% **	31.2%***	9.3%	15.6%*
Styrene and noise (N>85 dB)	none	9.5%	none	19.7%*	21.9% ***	32.0%***	none	20.8%***

*Significantly higher proportions than expected compared to the median of the Swedish database[18]. *P<0.05, **P<0.01, ***P<0.001

Table 3: Means and standard deviations of outcome measures in different auditory tests. Significant differences between controls and exposed groups are indicated

Group	PMTF			CRA		Interrupted speech			Speech in noise	
	Brief tone 4kHz threshold (dB SPL)	top of peak threshold (dB S/N)	top of valley threshold (dB S/N)	Latency right ear (ms)	Latency left ear (ms)	Mean % correct answers	% of group above 93%	% of group below 78%	dB S/N ratio	% above -7.8 S/N
Controls	37.0±11.1	18.8±5.6	12.5±6.6	134.5±22.2	134.8±20.4	90±13,4	47	6	-7.5	92
Noise	37.4±12.9	17.0±5.2[a]	11.3±5.8	141.0±20.2	142.5±25.8[a]	87±18.3	51	14	-5.3[a]	42[a]
Styrene (N<85 dB)	40.2±16.7	18.2±5.9	12.7±6.3	144.6±22.8[a]	136.5±20.2	85±13.5	32[a,b]	20[a]	-5.6[a]	70[a,b]
Styrene and noise (N>85 dB)	42.6±17.4[a,b]	18.3±5.3	12.4±6.6	138.2±24,2	147.7±25.5[a]	88±9.3	35[b]	17[a]	-5.3[a]	82[b]

PMTF = Psycho-acoustical modulation transfer function, CRA = Cortical response audiometry, S/N = Signal-to-noise ratio; [a] = $P < 0.05$ compared to control group, [b] = $P < 0.05$ compared to noise group.

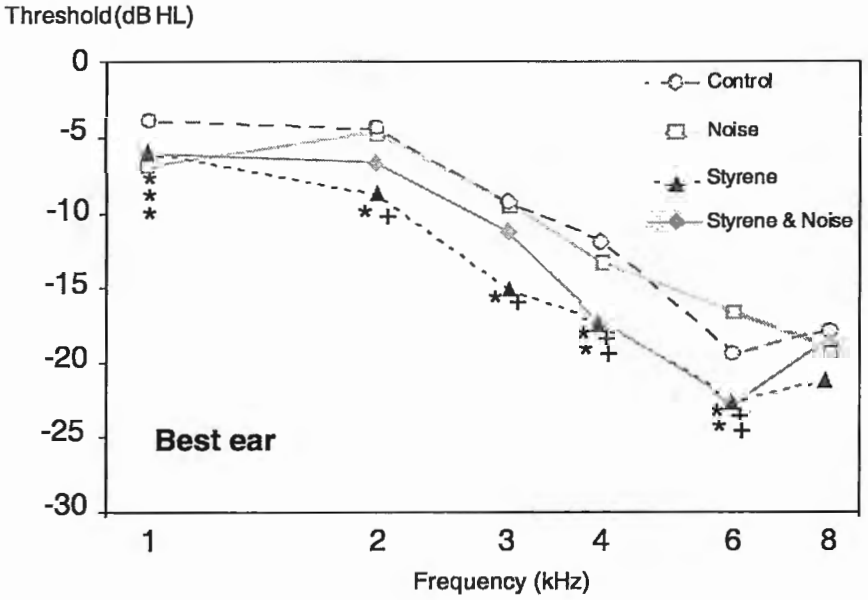

Figure 1: Group mean hearing levels of pure-tone audiometry (dB HL) for the better ear at frequencies between 1 and 8 kHz. Asterisks (*) indicate frequencies that met the significance level criterion ($P<0.05$) when exposed groups were compared to control workers and the plus sign (+) indicate frequencies that met the significance level criterion ($P<0.05$) when styrene-exposed groups were compared to noise-exposed workers.

three "exposed" groups. (Asterisk indicates Greenhouse-Geisser correction for degrees of freedom).

The mean DPOAE at higher levels (55-70 dB) was significantly correlated to the hearing thresholds at 4 kHz in the best ear of the controls and the group exposed to noise. The groups exposed to styrene did not show correlation with the hearing thresholds.

CRA

CRA was measured in both ears but no significant difference was found between the ears. A significant effect of exposure group on the latency of the cortical evoked response ($F(3, 263) = 3.48$, $P = 0.016$; MSE = 360.70) was seen. This observed significant effect permitted a post-hoc analysis to be performed on the data. Tukey's HSD carried out on the means for the four exposure group levels revealed a significant difference on the cortical response latency scores between the control and the exposed groups ($P < 0.05$; Table 3). The latency of the CRA was also correlated to the current noise exposure ($r = 0.146$, $P = 0.018$; $N = 266$).

Speech tests

Interrupted speech

The mean scores of the interrupted speech test were not significantly different between the groups. A tendency towards a lower score was seen in the group exposed to styrene alone compared to the controls ($P<0.06$). The group scores were compared to reference values using the classification criteria of normal values being above the median of 93% correct responses and of abnormal values at 78% and below. A significant lower percentage of both groups exposed to styrene were found above 93% ($P < 0.05$) and a significant higher percentage of both groups exposed to

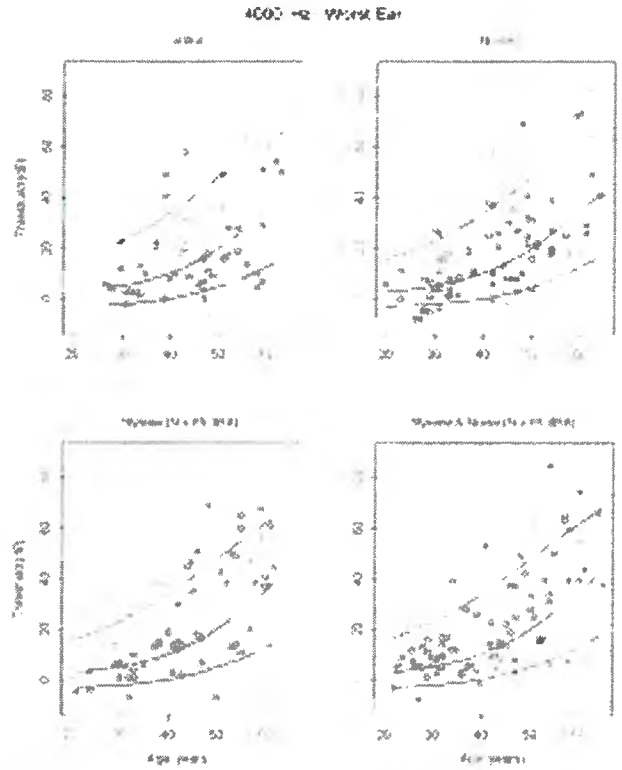

Figure 2: Age-related individual thresholds of pure-tone audiometry (circles, dB HL) of the worst ear at 4000 Hz are shown in separate graphs for different groups. Lines show the 10th, the 50th and the 90th percentiles of an otological unscreened, non-occupational noise-exposed population (n=603; age 20-79 years) from Sweden[18].

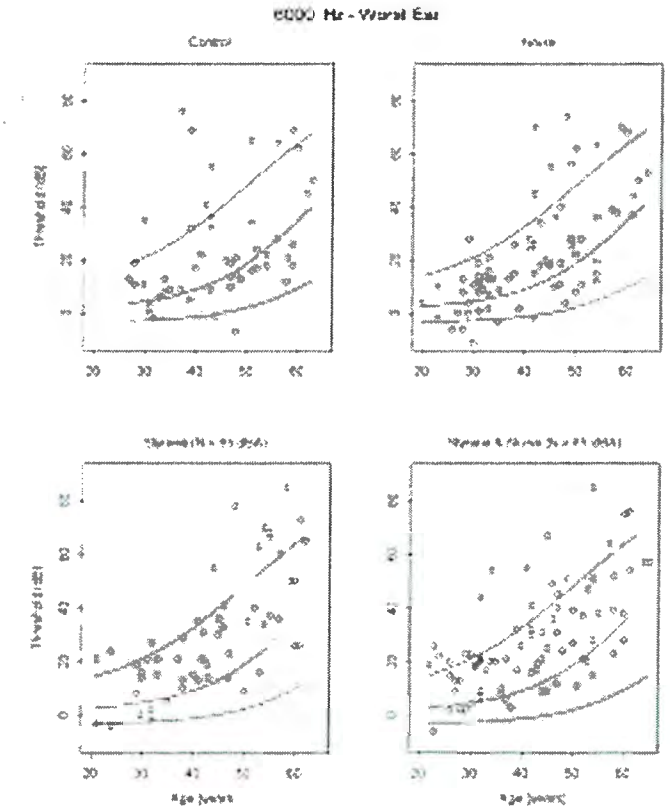

Figure 3: Age-related individual thresholds of pure-tone audiometry (circles, dB HL) of the worst ear at 6000 Hz are shown in separate graphs for different groups. Lines show the 10th, the 50th and the 90th percentiles of an otological unscreened, non-occupational noise-exposed population (n=603; age 20-79 years) from Sweden[18].

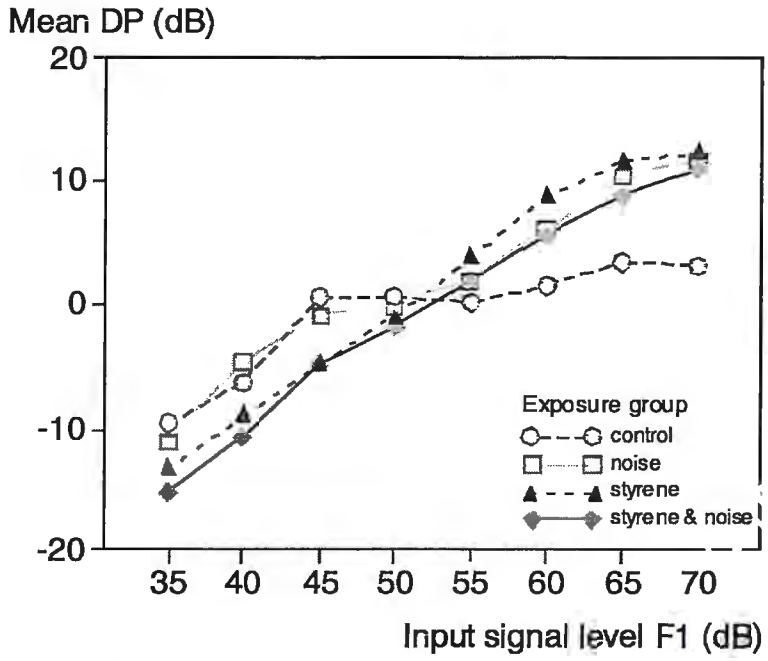

Figure 4: Mean distortion product (DP) scores for the four exposure groups, plotted against input signal level (F1). Note that at low input signal levels (up to 50 dB), control and noise groups have higher DP levels compared to the two groups exposed to styrene, while, above 50 dB, the control group levels off in contrast to all three "exposed" groups.

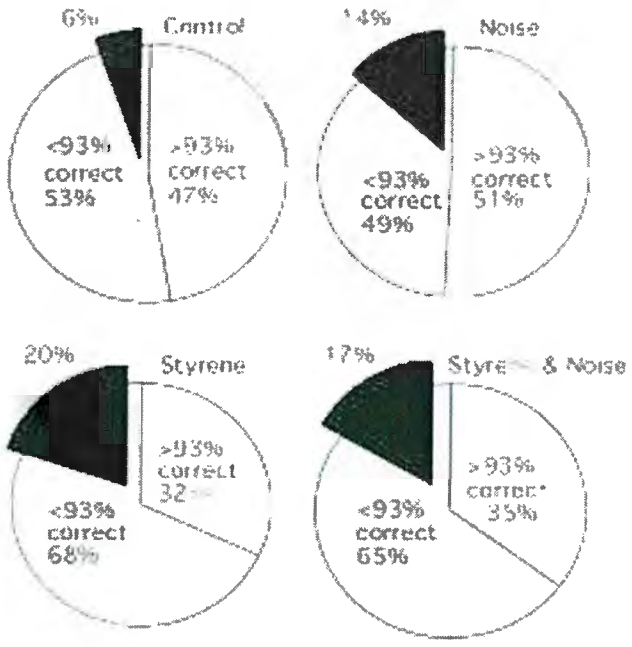

Figure 5: Interrupted speech test. The mean of correct responses is 93% in a normal population and scores below 78% (= mean minus 3 standard deviations) is considered abnormal[14].
Shown is the percentage (%) of each group that showed scoring values above (white) and below (grey) the normal mean of 93% correct responses. Black segments show the percentage (%) of each group with scoring values below 78% correct responses. A significantly higher percentage that showed scoring values below both 93% and 78% was found in the styrene group and in the styrene and noise group compared to the control group.

styrene were found below 78% ($P < 0.05$) as seen in Figure 5 and Table 3. A significant correlation was seen in the groups exposed to styrene between the cumulative styrene exposure in the evaluated company and a lower score in the interrupted speech (r=-0.190, $P<0.023$; N = 143). The result of interrupted speech test was also correlated to the speech in noise test (r = -0.378, $P <0.001$).

Speech recognition in noise

There was a highly significant effect of group on the signal to noise (S/N) ratio of the speech-in-noise test (F (3, 278) = 7.85, $P <0.001$; MSE = 8.71). A post-hoc test was performed on the S/N data. Tukey's HSD revealed a significant difference between the control condition and the three exposed conditions ($P<0.005$; Table 3). The same significant results were found for all exposed groups when they were compared to normal reference values of -7.8 S/N. The results of the speech in noise test were correlated to the current noise exposure (r = 0.124, $P =0.037$; N = 281).

Discussion

In the present investigation, styrene has been shown to affect the auditory system of exposed workers, despite the low levels of measured styrene at the studied workplaces. This finding confirms similar results reported in previous publications.[7,9,19] In 2002, the first stage of this study reported a higher prevalence of high-frequency hearing loss in the groups exposed to noise and styrene simultaneously (48%) and exposed to styrene alone (47%), compared to other groups: 33% in the control group and 42% in the noise-exposed group.[10] In each group, mean thresholds at each frequency were calculated for each ear. Significantly poorer thresholds at 2, 3, 4 and 6 kHz were observed in the styrene-exposed workers in both ears, compared with either of the two groups not exposed to styrene.

Since the publication of the 2002 report, a database containing the hearing threshold levels of an otologically unscreened population in Sweden became available,[18] along with a new mathematical model of hearing threshold levels as a function of age. We used the new model to re-examine the audiometric data we had previously analyzed by earlier methods. The new analysis confirmed our previous conclusions, indicating that styrene exposure was associated with significantly poorer thresholds at several of the test frequencies.

As discussed in the first report on this study, styrene-exposed workers had statistically significant poorer pure-tone thresholds at the frequencies of 2, 3, 4, 6 and 8 kHz than the other studied groups. At the frequencies of 2, 3 and 8 kHz the group exposed only to styrene had poorer thresholds than those exposed to both agents. This observation might be the result of the variations in exposure histories and parameters. The group exposed to styrene alone was exposed to higher concentrations of the solvent than the group exposed to both agents and also had higher lifetime styrene estimates.

In the present report we have observed not only the occurrence of audiometric effects in the styrene-exposed groups, but also information on the site of the disorders and the potential contribution of different audiological tests in the detection of the styrene effects.

Control group

The prevalence of hearing loss in the non-exposed control group was above 30%, higher than expected. This prevalence of hearing loss may have been due to the fact that some controls were exposed to high levels of noise in their previous jobs. The mean tenure in these jobs was, however, not different from any of the exposed groups. The calculated life-time noise exposure was also lower among the controls compared to the exposed groups. The results from the control group show the difficulty of finding a suitable control group among blue-collar workers. To compensate for this lack, we also used comparison to other control materials and databases when they became available.

Retesting part of the population

Twenty percent of the retested workers showed a significant threshold shift in at least one test frequency. In only one individual was the greater loss of hearing loss seen in more than one frequency. The exposure measurements of styrene and noise in the workplace did not differ between the occasions. Studies investigating the possible use of significant threshold shift percentages as an evaluation criterion to measure the effectiveness of hearing loss prevention programs have reported that 3% to 6%[20] or 5% significant threshold shifts[21,22] are reasonable incidence rates, which can be met. Significant threshold shift incidence rates exceeding these percentages suggest that preventive practices in place are not effective.[23] These present findings suggest that the studied agents are still affecting the workers' hearing.

Location of damage

Peripheral auditory system effects of solvent exposure were detected not only through pure-tone audiometry, but also on the results of distortion product otoacoustic emissions. Exposure to styrene has been associated with disruption in outer hair cell function as shown by DPOAEs in animals.[24,25] These authors indicated that DPOAEs could be used to monitor the ototoxicity induced by styrene even though they cannot be considered as the most sensitive index of styrene cochlear pathology, when compared to evoked potentials. Reduced amplitudes of transient evoked and distortion-product otoacoustic emissions have also been reported among solvent-exposed workers.[26]

The results of the input-output function in this study show different results for the two groups exposed to styrene compared with the controls and noise exposed workers. At low input signal levels (35-50 dB) the styrene groups showed

lower DPOAE responses compared with controls and noise exposed; whereas, at higher input signal levels, the controls showed lower DPOAE responses compared to all exposed groups. Hypothetically, this finding could mean that styrene affects the outer hair cell (OHC) function. The function of OHCs and their role as the cochlear amplifier is supposed to play a greater role in the formation of the DPOAEs at lower stimulation levels than higher; whereas, other mechanisms, such as linear coherent reflection, have a greater influence on the responses at higher stimulation levels.[27] However, this hypothesis is very speculative since recent research shows that more sophisticated measurements than those used in this study are needed to detect differences in how the responses of the DPOAEs are produced.[27]

The PMTF-results, show the typical decrease of top values for the noise group, which is however not apparent in the group exposed to styrene and noise, possibly because of several non-readable values. The reasons for the un-readable values are so far unknown. The threshold for the brief tone used in the PMTF-measurement, was higher in both styrene exposed groups. However, the brief tone threshold has only moderate correlation to OHC-loss and other mechanisms seem to be involved. The PMTF-test has been shown to be sensitive to OHC damage in earlier studies,[28] even if this was not evident here.

The interrupted speech test and the evoked cortical responses to frequency glides (CRA) are relatively insensitive to peripheral auditory lesions, but did indicate retrocochlear and central dysfunction. The CRA results indicated statistically significant differences between the groups, but the group differences were not clinically meaningful since there was a lot of overlap in the mean response times and standard deviations. Still, they might be helpful in the diagnosis of individual cases.

Clinical studies of workers with a long history of solvent exposure and a diagnosis of psycho-organic syndrome have detected significant abnormalities in central test results.[11,29-31] Also Laukli and Hansen[32] found abnormal results only in the central tests when using an extensive test battery for evaluating auditory effects after occupational exposure to solvents. In the present investigation, abnormal results of the interrupted speech and also the speech in noise test were shown to be associated with styrene exposure. Various methods of distorted speech have been used to test the central hearing pathways and the auditory cortex. Korsan-Bengtsen[14] showed that the most efficient distortion for this purpose is to interrupt the speech signal with 7 interruptions per second. Tests of interrupted speech are known to be sensitive to cortical lesions; however, brainstem lesions can also produce reduced scores.[14]

Occupational studies conducted on the ototoxic solvent toluene suggest a retrocochlear or central auditory pathway involvement in some of the hearing disorders observed, based on the results of acoustic reflex decay test.[33,34] Pitch Pattern sequence and duration pattern sequence also have shown to be sensitive tests for the detection of the central effects of occupational exposures to organophosphates and pyrethroid insecticides.[35] These tests are easy and fast to administer. From the clinical and research point of view, the use of tests with non-verbal material can be advantageous. Language, educational level and regional differences will not influence test performance.

The combined results of the tests performed in the present study suggest a location of the styrene damage both in the peripheral and the central portions of the auditory system.

Central auditory tests involving verbal responses require a number of neural functions such as attention, intensity or pitch discrimination, recognition, immediate memory and memory for sounds. The alterations observed in the present study offer clues to the type of the damage incurred in the individuals exposed to solvents. It is conceivable that the cortical area that supports language ability in the temporal-parietal region of the left hemisphere may be affected, as has been previously suggested.[36-38] However, these tests do not give information on exact sites or which hemisphere was affected. Thus, the issue of lesion site remains open for future studies.

Usefulness of test battery to complement pure tone audiometry

Several solvents commonly used in the workplace have been shown to have ototoxic properties. Noise is often present in occupational settings and it can be challenging to distinguish the effects of these physical and chemical agents.

The majority of investigations on occupational hearing loss have relied on averaging pure-tone thresholds to assess noise effects on auditory function. To investigate the effects of chemical exposure, this traditional approach may not be sufficient. Approaches that are more robust indicators of the risk posed by occupational chemical exposures on hearing include (1) classification of audiometric results using specific criteria and subsequent estimation of prevalence or incidence rates and (2) relative risk from analyzing hearing as a binary variable (normal vs. high frequency hearing loss). Some examples of these alternatives have been discussed previously by Morata and Lemasters.[39] The prevalence of hearing loss among groups with different exposure conditions should be examined even if audiometric thresholds, by themselves, do not allow for easy identification of the effect of chemicals on hearing and especially when pure-tone audiometry is the only available test.

Audiometric findings associated with exposure to several solvents reveal mild to moderate hearing losses. However, despite a mild audiometric effect, the hearing loss from solvents may significantly impact an individual's ability to communicate, especially considering that the solvents affect more than the peripheral auditory system. To better

understand and detect the effect of these chemicals and to differentiate effects of chemicals from effects from noise, it is necessary to identify the audiological tests that are sensitive to these effects.

In the present study, even though some abnormalities were noted on the DPOAE, PMTF and CRA testing, the most sensitive tests to the effects of styrene were interrupted speech and speech recognition in noise. These tests are easy to administer, non-invasive and allow for a distinction from the effects of noise and therefore, are recommended for use with solvent-exposed populations.

Electrophysiologic tests, such as the auditory brainstem response (ABR), CRA or event-related P300 potential, have been used in clinical investigations of the effects of industrial chemicals.[32] These tests require the use of more specialized equipment, extensive setup and test time and technical expertise. For these reasons their use in occupational studies may be unfeasible by both time and cost constraints. In the face of such constraints, the use of behavioral tests such as monaural low-redundancy speech tests, dichotic speech tests, temporal resolution or patterning tests and binaural interaction tests is suggested.

Conclusion

The results of the present study show that occupational exposure to styrene affects the auditory system even when the exposure levels are low. The audiometric test-battery showed that both the peripheral and the central auditory system were affected by these exposure conditions. These results show the need to make workers and the occupational health community aware of the potential risks of chemically induced hearing loss and to recommend the inclusion of chemical-exposed workers in hearing loss prevention programs, even when the noise levels are below recommended exposure limits.

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