

COGNITIVE EVOKED POTENTIALS VEP P300 IN PERSONS OCCUPATIONALLY EXPOSED TO LOW CONCENTRATIONS OF TOLUENE

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Cognitive evoked potentials VEP P300 were examined in 49 workers at printing press occupationally exposed to low concentrations of toluene for averagely 20 years, and in 59 control subjects. The exposure level of toluene was evaluated in randomly selected subgroups comprising 36 exposed and 27 control subjects. The concentrations of toluene were measured in peripheral blood on Wednesday morning before entering the work area, while the hippuric acid in urine was measured before and after entering the work area. The results show that the absolute number of exposed subjects displaying lower amplitude of cognitive wave VEP P300R and prolonged latency of the accompanying spontaneous wave VEP P300F was significantly greater than that of the controls.

Key words:
encephalopathy, neurotoxicity, organic solvents

When organic solvents are used in industry, apart from their technical characteristics, the important factor to be taken into account is the safety of the people exposed (1, 2). However, reports of adverse effects of toluene on the central nervous system in the occupationally exposed have been published for the last 15 years (1, 3-8). The most significant health problem, as determined by psychological testing, was the lowered efficiency of cognitive function. Apart from general conclusions, about which the majority of authors agree, the diversity of results substantially limits their application (3-5).

The aim of this study was to assess global cognitive possibilities by determining VEP P300 evoked potentials which show a particular correlation with cognitive functions (9-11). Our study of long-term occupational exposure to toluene was intended to achieve a far greater reproducibility of the results, that is, to substantially reduce the influence of subjective factors as a fundamental disadvantage of psychological testing (4, 9).

SUBJECTS

A group of 49 workers on a printing press and a group of 59 controls (employed in the production of metal frames and occupationally not exposed to neurotoxic agents) were examined for cognitive VEP P300 evoked potentials. Toluene has been used in printing as a solvent for printing inks and as a rinse for rollers for the last 30 years. For that entire period, the technology, workshops, ventilation, the number and type of related jobs has not changed. The fluctuation of the workforce was insignificant and most of the workers in both groups spent their entire work life at the same position. The mean age of the exposed workers was 42 years (range 24-43, SD=6.6) and of the control workers 43 years (range 23-43, SD=8). Both exposed and control workers had been in the service for averagely 21 years (range 3-33, SD=7, and 2-33, SD=8, respectively). There were 3 women in the exposed group, and 5 in the control group. Both groups had averagely 11 years of education.

METHODS

The level of exposure to toluene in both groups was calculated by measuring the concentrations of toluene in peripheral blood and of the hippuric acid in urine, corrected according to the amount of excreted creatinine. The measurements were carried out in the clinic belonging to the printing office on Wednesday before the workers entered the work area. The urine concentration of hippuric acid was determined again on the same day after the shift.

Toluene was determined by »head space« gas chromatography as described in *Angerer* (12), and hippuric acid as described in *Buchet and Lauwerys* (13). The analysed subgroups consisted of 36 exposed and 27 control, randomly chosen subjects.

The questionnaire completed by the clinic's physician provided data related to smoking, alcohol and coffee consumption, medicaments (analgesics, sedatives and other), head and neck injuries, loss of consciousness, and the use of general anaesthetics during operations.

VEP P300: The P300 wave was determined in all subjects by visual stimulation with a structured stimuli chessboard on a Brain Imager (Neuroscience-Medilog, 1988). We used two-dimensional structured stimuli which changed randomly each second, while for the less frequent one-dimensional stimuli the subject's attention was held by counting. The angle at which the subject viewed the smaller dimension of the structured stimuli chessboard with both eyes was one degree, and the bigger square, two degrees. The contrast between the light and the dark square was 92% and the constant light in the examination room maintained an average intensity of 0.05 cd/m². The portion of rare stimuli in the total number of stimuli was 35% and the examination was concluded when the number of more frequent stimulations reached 100. The examination was repeated if the counting error exceeded one and if this occurred again, the subject was excluded from the study.

Responses were received through a Cz electrode, and the greatest positive deflection on the ordinate was taken as the P300 wave which occurred 260 to 450 ms after the stimulation was initiated. The lower limit of filtering all responses was 1.05 Hz and the upper limit 80 Hz. The registered responses were divided in two groups. The first group of responses referred to stimulation during which the subject's attention was held by counting (less frequent stimulation), called VEP P300R wave, that is, the actual cognitive evoked potential P300. The second group of responses referred to stimulation during which the subject's attention was not held by counting (more frequent stimulation), called VEP P300F wave, that is, accompanying spontaneous P300 wave (Figure 1). The following parameters were determined by waves P300R and P300F: the time of commencement, maximum amplitude achieved (latency), the

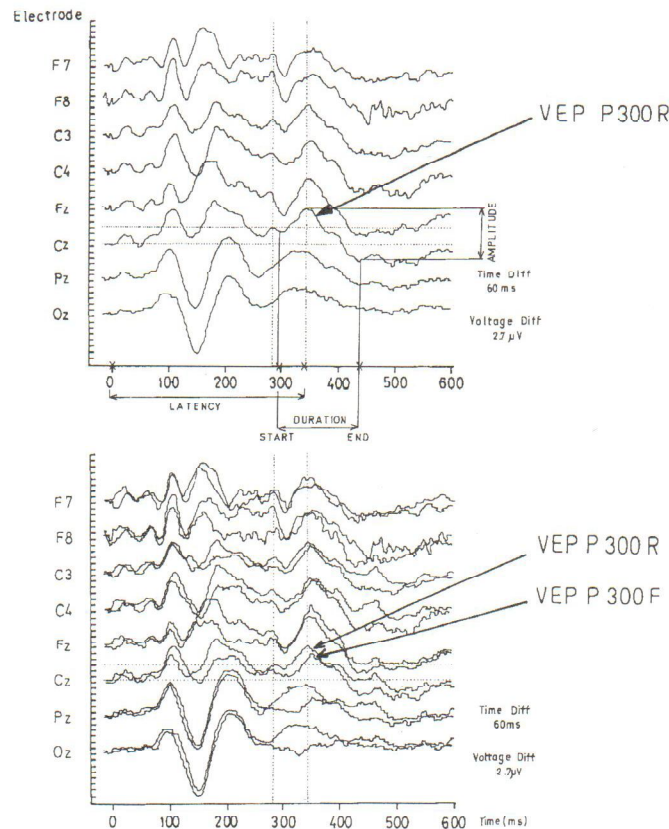


Figure 1 Example of a rare case among workers exposed to toluene (V.M., 48 years old, exposed 25 years) with both latency and amplitude $P300R < P300F$. More frequent latency among workers was $P300R > P300F$.

end and the total duration in milliseconds, and the maximum amplitude as the distance on the axis between peaks of the respective positive and negative wave.

Numeric data were statistically analysed by Wilcoxon Mann-Whitney U-Test (WMW) when the distribution was abnormal, and by chi-square and Student's t-test when the distribution of data was normal. The differences were considered significant at level $P \leq 0.05$.

RESULTS

The answers to the questionnaire showed that there were no differences between the subjects exposed to long-term low concentrations of toluene and the control subjects with regard to age, years of work, education, alcohol and coffee consumption, smoking habits, head injuries and intake of specific medication. In both the exposed and the control group, practically the same number of persons reported daily alcohol consumption (N=40 and N=44, respectively) or at least four times a week, the same number of years of such drinking (12.7 ± 8.6 and 11.7 ± 9.8 , respectively), and the same average daily amount of alcohol consumed expressed as the equivalent of pure alcohol in grams (78.6 ± 32.5 and 76.3 ± 38.6 , respectively). Smokers were equally distributed in the exposed and the control group (N=28 and N=29, respectively), with similar years of smoking (16.3 ± 11.1 and 16.1 ± 12.2 , respectively), and the daily consumption of cigarettes smoked (24.6 ± 13.2 and 25.3 ± 12.2 , respectively). Similarly, the number of subjects who drank coffee daily (N=39 exposed and N=48 controls), and the number of years with daily consumption of coffee (3.3 ± 1.8 , exposed and 2.2 ± 1.6 , controls) hardly differed.

No significant differences were found between the groups with regards to the number of head injuries with loss of consciousness (N=9 exposed and N=4 controls) or without disturbed consciousness (N=9 exposed and N=11 controls). Furthermore, the groups did not display significant differences in the number of persons taking analgesics (N=10 exposed and N=12 controls) and sedatives (N=10 exposed and N=12 controls), or in the number of administered general anaesthetics (N=17 exposed and N=14 controls).

Table 1 Concentrations of toluene in blood on Wednesday morning prior to work, and hippuric acid and ortho-cresol in urine on Wednesday morning prior to and after work

	Exposed N=36	Control N=27
Wednesday morning before work		
Toluene in blood (mg/L)	$0.036 \pm 0.025^{**}$	0.0096 ± 0.0037
Hippuric acid in urine (g/g creatinine)	$0.426 \pm 0.262^*$	0.338 ± 0.224
Wednesday after work		
Hippuric acid in urine (g/g creatinine)	$0.485 \pm 0.261^{**}$	0.223 ± 0.120

The results are presented as means and standard deviations. Significant differences between examined groups: $^{**}P < 0.01$; $^*P < 0.05$.

The concentrations of toluene in peripheral blood and of hippuric acid in urine are presented in Table 1. The concentration of toluene in peripheral blood on Wednesday morning before workers entered the work areas was thirty times greater in the exposed workers than in the controls. Toluene purity was 98% and benzene concentration 0.3%. The concentration of hippuric acid in urine in the subgroup of exposed subjects was significantly greater than in the controls with a trend to increase with the closing of the workday.

Table 2 Cognitive wave VEP P300R registered by Cz electrode

	Exposed N=49		Control N=56	
	Median	Range	Median	Range
Start (ms)	324	196-402	324	250-388
Peak (ms)	369	296-424	368	334-478
End (ms)	416	350-534	427	346-610
Amplitude (μ V)	9.3	2.5-20.0	8.7	1.7-15.2
Duration (ms)	90	12-314	98	8-306

Results are presented as median values and their range.

The examined groups displayed no significant differences in the following parameters of waves VEP P300R and VEP P300F: time of commencement, maximum amplitude (latency) reached, the end and the total duration (Tables 2 and 3). No significant differences were found in the amplitudes of wave VEP P300R (Table 2). However, the amplitude of wave VEP P300F in the control subjects was significantly greater (Table 3).

Table 3 Accompanying spontaneous wave VEP P300F registered by Cz electrode

	Exposed N=48		Control N=56	
	Median	Range	Median	Range
Start (ms)	322	176-478	337	78-474
Peak (ms)	364	308-516	376	336-480
End (ms)	411	356-578	415	352-710
Amplitude (mV)	8.6**	2-17	7.5	2.37-15
Duration (ms)	92	21-248	76	8-254

The results are presented as median values and their range. Significant differences between examined groups:
**P<0.01.

Within the exposed group, a significantly higher absolute number of the subjects displayed smaller amplitude of wave VEP P300R than the amplitude of the accompanying wave VEP P300F (Table 4, upper part). At the same time, a significantly higher

absolute number of the subjects displayed greater latency of wave P300R than the latency of the accompanying wave VEP P300F (Table 4, lower part).

Table 4 Relation of P300R and P300F wave amplitudes and wave latencies

	Exposed N=48		Control N=56	
	P300R>P300F	P300R<P300F	P300F>P300F	P300R<P300F
Amplitude*	N=19	N=29	N=36	N=19
Latency*	N=33	N=15	N=14	N=42

The results are presented as absolute numbers (N) of subjects with the observed measured paramotor. Significant differences between examined groups: *P<0.05.

DISCUSSION

Correct interpretation of the results of evoked potentials requires knowledge of as many factors that may affect a subject as possible (14). That is why we collected data and performed tests for alcohol and coffee consumption, smoking, head and neck injuries, impaired consciousness and medications.

As we found no significant differences between the groups for the above factors, the interpretation of the results proved particularly valuable. We previously stressed the attitude of subjects to alcohol as several investigations on the influence of long-term exposure to low concentrations of toluene proclaimed alcohol to be the basic etiological factor with regard to the changes found (15-17). Uniform methods for evaluation of exposure, particularly occupational, are still to be agreed, not only in the case of toluene but for other organic solvents as well (1, 2). In this study, we decided to use biological markers in order to encompass all possible means of intake, particularly through the skin (18). Since the workers were exposed to low concentrations of toluene, that is to low bioaccumulation, we estimated that determining biological markers of toluene exposure on midweek would best approximate the values of the whole working week (1, 2).

Hippuric acid in urine is excreted in varying amounts in people not exposed to toluene as a metabolite of benzoic acid. Our results from measuring hippuric acid in urine proved it a valid test for evaluating low levels of exposure to toluene, although only on a group basis (19). The design paradigm-oddball task in this investigation is based on two types of structured light stimuli which should be as similar as possible in order to spontaneously provoke corresponding evoked late potentials over the vortex (Cz electrode) without mental engagement by counting. The difference obtained between them, after holding subject's attention to one of the forms by counting, can therefore be mainly attributed to his mental engagement (9).

When compared to the control group, it would appear that the occupational exposure to low concentrations of toluene for an average of 20.6 years in printing

press workers with averagely 0.036 mg/L toluene in peripheral blood found on Wednesday morning before entering the work area had no essential effect on the parameters of so-called cognitive evoked potentials VEP P300R. However, there is a significant effect on the parameters representing the relation of this wave to so-called spontaneous wave VEP P300F. Namely, the occurrence of a significantly greater absolute number of those in the exposed group with lower value of amplitude of P300R wave than the accompanying P300F wave and in whom the same wave occurred later in relation to the accompanying P300F wave, prompted the following speculation: chronic exposure to low concentrations of toluene can cause reduced amplitude and delayed occurrence of the evoked potential over the secondary sensitive brain cortex during mental engagement compared to a spontaneously evoked potential similarly provoked but without mental engagement. Presumably, a mechanism for desynchronisation of the potential generator exists, reflecting the effect of chronic exposure to toluene on the intracranial structure, particularly the extended brain stem and hypothalamus (11, 14).

The question is whether these two phenomena are the reason for the observed reduction in efficiency of cognitive function in the examined subjects occupationally exposed to toluene.

CONCLUSIONS

Chronic exposure to low concentrations of toluene in subjects with averagely 0.036 mg/L of toluene found in peripheral blood on Wednesday morning before entering the work area did not significantly alter parameters of cognitive evoked potential VEP P300.

In the above conditions, the parameters changed significantly showing the relationship with other similar spontaneous potentials not connected with mental engagement. The reduction in amplitude occurred and the cognitive evoked potential was deferred in relation to a similar spontaneous potential.

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Sažetak

KOGNITIVNI EVOCIRANI POTENCIJALI VEP P300 U OSOBA PROFESIONALNO IZLOŽENIH NISKIM KONCENTRACIJAMA TOLUENA

Kognitivni evocirani potencijali VEP P300 procjenjivani su u 49 tiskarskih radnika izloženih profesionalno niskim koncentracijama toluena prosječno 20 godina i u 59 kontrolnih osoba. Ispitivane skupine nisu se razlikovale po dobi, godinama radnog staža i naobrazbi. Među skupinama nije bilo razlika u navikama pijenja alkoholnih pića i pušenja cigareta, u uzimanju specifičnih lijekova (analgetika i sedativa), u broju podvrgavanja općoj anesteziji zbog operativnog zahvata, kao ni u broju ozljeda glave i vrata ili broju gubitaka svijesti. Svi ovi pokazatelji mogu utjecati na rezultate mjerenja evociranih potencijala i njihovo je procjenjivanje nužno u ispitivanju učinaka specifičnih onečišćivača kakav je toluen. Razina izloženosti toluenu procijenjena je u slučajno izabranih podskupina od 32 izložena radnika i 27 kontrolnih ispitanika. Izmjerene koncentracije toluena u perifernoj krvi i hipurne kiseline u mokraći ujutro u sredini tjedna (u srijedu) prije nego li su radnici ušli u radni prostor i

koncentracije hipurne kiseline u mokraći nakon radne smjene istoga dana bile su značajno više u izloženoj skupini. Među ispitivanim izloženim radnicima nađen je značajno veći broj onih koji su imali kognitivni val VEP P300R niže amplitude i duže latencije u usporedbi sa spontanim valom VEP P300F.

Ključne riječi:
encefalopatija, neurotoksičnost, organska otapala

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