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Acute effects on the human EEG after an external exposure
to 200 ppm methanol

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Abstract Objectives: Even low concentrations of organic solvents may cause acute effects on the human central nervous system. The German MAK (threshold limit value) of methanol is 200 ppm. The aim of this study was to investigate whether acute exposure to 200 ppm methanol causes adverse effects, measured by EEG, and moreover, whether it is possible to differentiate between sedative and excitatory effects with this method. Methods: Twelve healthy subjects were exposed for 4 h to 200 ppm and to 20 ppm (control) in an exposure chamber in a cross-over design. The EEG was recorded before (reference) and at the end of each exposure with, the subject’s eyes closed and opened and during a choice reaction test (color word stress test). Spectral power was calculated by fast Fourier transformation. Subjective symptoms and effects of blinding with 20 ppm methanol were assessed by questionnaires. Results: The study was a single-blind one. During subjects’ exposure to 200 ppm, their scores for prenarcotic and irritating symptoms were not different from controls. In the closed-eye condition of subjects, the spectral power of the $\theta$-band and of some electrodes of the $\delta$-band was significantly less at the end of exposure to 200 ppm, than that of controls. In the open-eye condition and during the color word stress test no significant changes were found. Conclusion: The changes in the $\theta$-band suggest a slight excitatory effect of 200 ppm methanol. The effect was weak, as scores of acute symptoms did not change. With respect to our results, it is not necessary for the MAK value to be decreased.

Key words Methanol · EEG · Threshold limit value · Color word stress test · Exposure chamber

Introduction

Methanol is a solvent used worldwide. Different mixtures of solvents, e.g. lacquers and thinners, may contain methanol. Furthermore, it could become an important motor fuel. In the evaluation of threshold limit values for work places, both acute and chronic effects have to be considered. In this study we focused on possible acute effects. Acute exposure to solvents in general may cause prenarcotic symptoms, if concentrations are sufficiently high. Concentrations in the magnitude of threshold limit values for workplaces may impair neurophysiological and/or neuropsychological functions [12]. However, low doses of certain solvents, e.g. ethanol, may have an excitatory effect on the central nervous system. Therefore, we looked for a sensitive neurophysiological method to detect and to characterize acute effects. The quantitative analysis of EEGs has proved to be a suitable method for the detection of slight acute effects of medications [19] and of substances used at workplaces, such as organic solvents and organophosphorus pesticides [34, 43]. Furthermore, it can give hints on the mode of action of a substance [19]. Therefore, we chose the quantitative analysis of EEGs to study acute effects of methanol on humans. Inhalation is the most common route of entry in an occupational setting. As the German threshold limit value (MAK) is 200 ppm methanol, we exposed voluntary subjects to 200 ppm for 4 h in an exposure chamber. In contrast to intoxication with optic neuropathy, due to oral ingestion of large amounts of methanol which is metabolized to formate, being the main toxic agent, acute exposure to 200 ppm methanol...
does not increase serum concentrations of formaldehyde and of formate [24, 31, 36, 40]. So possible effects could be related to the action of methanol itself.

We questioned whether acute exposure to 200 ppm methanol evokes adverse effects, which would necessitate the lowering of the threshold limit value. From a toxicological point of view, we were interested in the mode of a possible effect.

**Methods**

**Experimental design**

Twelve subjects were exposed for 4 h to 200 ppm and to 20 ppm (control) methanol in an exposure chamber according to a crossover design. The interval between the two sessions was 1 week. 20 ppm were chosen in order to blind test subjects and staff. Preliminary experiments indicated that 20 ppm methanol could be recognized by persons with normal olfaction. We did not use a non-toxic odorant substance like peppermint oil (e.g. [23, 41, 43]) to blind, as peppermint oil itself may cause changes of the EEG [3]. The effect of blinding was investigated by questionnaires. The EEG was recorded in the chamber before exposure (reference) and after 3.7 h of exposure. Subjective symptoms were assessed by questionnaire before, 2 h after and 4 h after exposure.

**Subjects**

Subjects were 12 healthy male, non-smoking, right-handed students (mean age 26.8 ± 2.1 years). Handedness was assessed with the Edinburgh Inventory [35]. Subjects did not take any medications. A preliminary examination included occupational and past-medical history, physical examination, visual acuity, electrocardiogram, spirometry, blood count, determination of γ-glutamyl transpeptidase, and erythrocyte sedimentation rate. Normal smell acuity was assessed by olfactory function testing with the “Sniffin’ Sticks” [21, 22].

Subjects were paid to participate. Prior to the study, we obtained written informed consent from every subject. The study was performed in accordance with the ethical principles of the Declaration of Helsinki (version of Hong Kong of 1989). The protocol was approved by the local ethics committee.

**Exposure**

The exposure to methanol (methanol 99.8% p.a. A.C.S. Merck, Darmstadt, Germany) was performed in an 18 m³ exposure chamber. Concentrations were 203.5 ± 2.5 SD ppm and 20.3 ± 3.8 ppm methanol, respectively, monitored with an infrared analyzer (Miran 980, Foxboro Analytical, South Norwalk, Conn., USA). Temperature was kept at 20.4 ± 0.7 °C. Humidity was 48.1 ± 2.6%. There was a change of air six times per hour. Blood samples were taken every hour for the determination of methanol concentrations. However, measurements could not be evaluated because of analytical difficulties.

**Evaluation of efficacy of double-blind procedures**

Test subjects were informed that they would be exposed to two different concentrations of methanol, not exceeding the German MAK value, in random order. After the second session, they were asked by questionnaire at which experimental session had they been exposed to the higher concentration. They checked their degree of certainty on an ordinal scale from 1 (not at all) to 5 (absolutely sure). They were also asked for a short explanation. Staff filled out an analogue questionnaire after each experimental session.

**EEG**

Subjects were separated from staff by a curtain, and were monitored with a video-system. EEGs were recorded with electrode caps (Electro Cap, Co., USA) [6] for 5 min while subjects closed their eyes, for 5 min with open eyes, and for 6 min during the color word stress (CWS) test of the Swedish Performance Evaluation System (SPES) [15]. The CWS is a choice reaction time task. It was applied to yield a comparable mental load for all subjects. The German words for red, yellow, white or blue were presented on a screen. The text could be written in any one of the colors. The task was to press a key as fast as possible when there was congruency between the meaning of the word and the color of the text. The interval between subsequent stimuli was 1.5 s. Proportion of critical stimuli was 75%. Reaction time and number of errors were registered in order to control the performance of subjects. Analog EEG signals, electrooculogram, and electrocardiogram signals were fed into a battery-powered amplifier (MediSyst, Linden, Germany), which was placed near the subject. In the amplifier (bandpass 0.25–70 Hz) all analogue signals were digitized (512 Hz/12 bit) and then transferred via an optic-fiber to a computer (CATEEM, MediSyst, Linden, Germany). The high input impedance of the amplifier (AC = 10 megaohms, DC = 20 megaohms) ensured a sufficient signal-to-noise ratio. The analogue signals were displayed on a screen for visual control. Test subjects were not kept alert by noise, when drowsiness patterns appeared in the record, as possible changes of vigilance should be investigated. Most artifacts (e.g. eyeblinks) were recognized automatically by a computer with adjustable sensitivity, and were eliminated from further analysis. After the experiments, remaining artifacts were eliminated by visual control without awareness of subjects and their exposure. Most of the time, some subjects had myograms in single leads when their eyes were open. Therefore, these epochs could not be eliminated and the β₁- and β₂-band at some electrodes were not analyzed. Common average reference was calculated from the signals of 16 electrodes measured against Cz, hence obtaining signals from 17 real electrodes against a virtual reference [25]. After smoothing, we averaged the signal of four consecutive data points, which gave an effective sampling rate of 128 Hz. Frequency-analysis was performed with a Hanning window and a fast Fourier transformation of epochs of 4 s. Power spectra were calculated with a spectral discrimination of 0.25 Hz. The frequency spectra were divided into six frequency bands (Δ = 1.25–4.50 Hz, θ = 4.75–6.75 Hz, χ₁ = 7.00–9.50 Hz, χ₂ = 9.75–12.50 Hz, β₁ = 12.75–18.50 Hz, β₂ = 18.75–35.00 Hz). Absolute power of the six frequency bands was calculated.

**Assessment of acute symptoms**

An SPES questionnaire [17] was administered before, 2 h and 4 h after exposure, by use of a German translation [42]. It contains 17 items related to irritation of mucous membranes, difficulties in breathing, and pre-narcotic symptoms. Subjects were requested to check off the degree of their symptoms on an ordinal scale from 0 (no symptom) to 5 (severe symptom). Three subjects received Liquifilm eye drops (Pharm-Allergan, Ettingen, Germany), an artificial lachrymal fluid which prevents frequent eye blinks, when their EEGs were recorded while their eyes were open. Therefore, the items concerning the irritating effects on the eyes were not evaluated for these subjects.

**Experimental procedure**

After having the preliminary examination, subjects took part in a training session. Recording of EEGs and SPES questionnaires were performed five times. Subjects were instructed to have breakfast at home and to avoid coffee, black tea and cola. They were told to avoid ethanol and food or beverages containing methanol [36] 24 h prior to experiments. During experimental days, subjects were asked what foods and beverages they had consumed in the 24 h prior to the experiment. Immediately before each exposure, a short