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Acute Effects of an Organic Solvent Mixture on the Human Central Nervous System

A. Muttray[1], P. Martus[2], S. Schachtrup[1], E. Müller[1], O. Mayer-Popken[1], J. Konietzko[1]

[1]Institute of Occupational, Social and Environmental Medicine, University of Mainz, Germany
[2]Institute for Medical Informatics, Biometry and Epidemiology, Free University Berlin, Germany (formerly Institute for Medical Biometry, Epidemiology and Informatics, University of Mainz, Germany)

Abstract:
Objectives: At workplaces, organic solvents are often used as mixtures. Nevertheless, there is limited knowledge of their acute effects on human central nervous system. Here we report the effects of a toluene-acetone mixture.
Methods: In a parallel design, subgroups of 12 healthy men each were exposed to a mixture containing 25 ppm acetone and 250 ppm toluene or to air (control) in an exposure chamber for 4.5 hours. Concentrations corresponded to the German TLV (TRGS 403). Concentrations of toluene and acetone in venous blood were measured by headspace gaschromatography. Subjects were sedentary. The following tests were performed before and at the end of exposure: Questionnaires, simple reaction time, vigilance, quantitative analysis of EEG with open and closed eyes and during the Color Word Stress test, and visual evoked potentials (VEP).
Results: Blood levels were 0.14 (± 0.04 SD) mg toluene/l and 5.43 (± 1.37 SD) mg acetone/l at the end of solvent exposure. Scores of neurotoxic and irritating symptoms were not elevated during solvent exposure. Exposed subjects performed as well as control subjects on the simple reaction time test and on the vigilance test, neither reaction time nor number of hits differed significantly. A general linear model on log transformed spectral power values showed insignificant changes in EEG. In the α_2-band an average reduction to 86 % was observed in exposed as compared to non exposed subjects with closed eyes, a reduction to 88 % in the θ-band with open eyes, and a reduction to 92 % in the θ-band during the Color Word Stress test. VEP P 100 latencies and amplitudes did not change.
Conclusion: The mixture consisting of toluene and acetone did not cause any adverse acute effect. With respect to EEG data, possible subclinical effects on central nervous system cannot be excluded.

Key words: Solvent exposure; reaction time; vigilance; EEG; visual evoked potentials

Abbreviations: CWS: Color Word Stress test; MAK: Maximale Arbeitsplatzkonzentration, the German threshold limit value for a toxic substance; SPES: Swedish Performance Evaluation System; TLV: Threshold limit value; TRGS 403: Technische Regel für Gefahrstoffe 403 (German threshold limit value for mixtures); VEP: Visual evoked potentials

Introduction

Mixtures of organic solvents are used for numerous processes in industry and craft. Even if working conditions have markedly improved in the last decade, there are still many workers being exposed to organic solvents. Mainly, the acute effects of single solvents or mixtures of two components were tested [5]. However, technical products often contain a number of different compounds. There is limited knowledge on the acute effects of commercially used mixtures. In a field study on painters, changes of solvent exposure were associated with the number of slips, trips and falls [10], probably due to acute central nervous system effects. We were interested in determining, if solvent mixture exposure within the German threshold limit values (TLV) may cause acute effects on the human central nervous system. We decided to investigate the effects of mixtures in an experimental chamber study under controlled conditions. Exposing subjects to a mixture of solvents does not allow us to determine the way of possible interactions of the single components. But the outcome corresponds to an overall effect, considering interactions if they had taken place. Furthermore, it is relevant for the practical use and for reviewing the threshold limit values. The study was planned together with the "Arbeitskreis Lösemittel des Hauptverbands der gewerblichen Berufsgenossenschaften" (expert group for solvents of the German federation of institutions for statutory accident insurance and prevention). Here we report the effects of a mixture containing toluene (25 ppm) and acetone (250 ppm) which was chosen for comparison with a commercial thinner (effects to be reported elsewhere). The thinner contained mainly toluene, acetone, and isopropanol, which is quantitatively metabolised to acetone. An external exposure to 400 ppm isopropanol is supposed to result in blood levels of 50 mg acetone/I and 7 mg isopropanol/l [23].

According to TRGS 403, an index I must not exceed 1:

$$I = \frac{C^1}{MAK_1} + \frac{C^2}{MAK_2} + \text{.....} + \frac{C^n}{MAK_n}$$

where: C = measured concentration of solvent 1, 2, ..., n
MAK = MAK-value e.g. the German threshold value for the particular solvent. Solvents must be only considered, if concentrations are higher than 10 % of their MAK-values.

Concentrations of toluene and acetone were at 50% each of the MAK values. Hence, the index according TRGS 403 was 1.

In former studies, neurobehavioral assessment of occupational relevant solvents in humans was performed mainly with psychophysiological tests [5]. Changes of performance are due to effects on neurochemical processes in the brain [18]. These can be investigated with the EEG. Recent research indicated that quantitative analysis of EEG is a sensitive method to detect subclinical effects of organic solvents [15, 16]. An acute exposure to 200 ppm methanol decreased the spectral power of the θ-band [15], indicating a subclinical excitatory effect. 200 ppm 1,1,1-trichloroethane caused acute changes in EEG indicating a slight sedative effect [16]. These results show that organic solvents have different modes of action. To be able to detect slight sedative effects, we additionally used a vigilance test for fatigue in the present study.

The aim of our study was to evaluate if mixtures can cause adverse effects, when concentrations of the mixtures meet the threshold limit values. We were also interested to identify mechanisms causing acute effects in the human central nervous system, by comparing possible EEG changes after solvent exposure with already known EEG profiles of various centrally acting pharmacons.

Methods

Experimental Design

Two subgroups of twelve healthy male subjects, each, were exposed either to a mixture of 25 ppm toluene and 250 ppm acetone or to sham (air) according to a parallel design. Subjects were assigned to the groups by chance. Though the used solvents are easily distinguished by their smell, it was decided not to blind subjects to the solvent smell by a substance like peppermint oil [11], which itself is a known trigeminal nerve irritant and may also cause interfering changes in EEG [2]. The subjects were informed to expect different intensities and qualities of odour. Exposure took place in an exposure chamber. All measurements before and during solvent exposure were taken in the chamber apart from the VEP, which were recorded in an adjacent room. Subjects were separated from the investigators by a curtain during the psycho-physiological tests (simple reaction time and vigilance) and neurophysiological (EEG and VEP) measurements. To reduce variability values obtained during exposure were related to baseline values measured before exposure.

Subjects

Subjects were healthy male, right-handed, non-smoking students and post-graduates. Most of them were social drinkers. Average daily ethanol intake of each subject was less than 25 g. Mean age of the exposed persons (n = 12) was 24.1 (± 1.8 SD) years, of the control persons (n = 12) 24.3 (± 2.5 SD). Handedness was assessed with the Edinburgh Inventory [17] and a handgrip dynamometer (Battendorf, Brussels), that measured the maximum voluntary contraction of each hand. Usually, strength of the dominating hand is higher. Subjects were on no medication. A preliminary examination included occupational and past-medical history, physical examination, visual acuity, colour vision (Ishihara plates), electrocardiogram, spirometry, blood count, determination of γ-glutamyl transpeptidase, erythrocyte sedimentation rate, and urine analysis. Subjects were paid for participation. Prior to the study, written informed consent was obtained from every subject. The study was performed in accordance with the ethical principles of the Declaration of Helsinki. The protocol was approved by the local ethics committee.

Exposure

The exposure to the mixture of 24.7 (± 2.4 SD) ppm toluene (p.a. Merck, Darmstadt, Germany) and 247 (± 4 SD) ppm acetone (Aceton purum ≥ 99%, Fluka, Buchs, Switzerland) and to sham was performed in a 18 m^3 exposure chamber for 4.5 hours. Concentrations were monitored with an infrared analyser (Miran 980, Foxboro Analytical, South Norwalk, Connecticut, USA). Temperature and humidity were constant at 21.3 °C (± 0.2 SD) and 46.4 % (± 0.5 SD) during solvent exposure and 21.2 °C (± 0.5 SD) and 48.4 % (± 1.3 SD) during exposure to sham. Venous blood concentrations of toluene and acetone were measured by headspace gaschromatography.

Subjective Ratings of Exposure Level

With a self-assessment questionnaire subjects rated the subjective level of exposure on an ordinal scale from 1 (no solvent detectable) to 5 (high solvent concentration) after the final measurement. After all tests were completed, they marked the degree of certainty of their exposure estimates on an ordinal scale from 1 (not at all) to 5 (absolutely sure). Staff filled in an analogue questionnaire after each experimental session.

Assessment of Acute Symptoms

A questionnaire of the Swedish Performance Evaluation System (SPES, [9]) was administered before, during and after exposure, using a German translation [20]. It contains 17 items related to discomfort, tiredness, irritation, and difficulties in breathing. Subjects were requested to check off the degree of their symptoms on an ordinal scale from 0 (no symptom) to 5 (severe symptom). Some subjects received Liquifilm® eye drops (Pharm-Allergan, Ettlingen, Germany), an artificial lacrimal fluid, to avoid frequent eye blinks when EEG was recorded with eyes open. Therefore, the items concerning the irritating effects on the eyes of these subjects were not evaluated. Also subjective well-being (tension as "relaxed … strained", tiredness as "awake … tired", complaints as "without complaints

… severe complaints", and annoyance as "not annoying … very annoying") was rated, using a German questionnaire of Seeber et al. [21]. Responses were evaluated according to an ordinal scale from 0 to 6.

Simple Reaction Time Test

The test stemmed from the Swedish Performance Evaluation System (SPES) [9]. The task was to press a key on a keyboard as quickly as possible when a red square was presented on a screen. A total of 96 stimuli were administered during 6 min at intervals between 2.5 and 5.0 s. The first minute served as practice, after which the performance capacity was assessed for 5 min.

Mackworth Clocck Vigilance Test

Figure 1a shows the principle of the vigilance test of the Wiener Testsystem® (Dr. G. Schuhfried, Mödling, Austria). The version called Quatember-Maly was only used to familiarise subjects with the principle of the test. The filled circle moves from one position to the next. It skips one field at varying intervals. Then, the subject has to press a key quickly. In preliminary experiments, the subjects exercised the version "Müggenburg" (Fig. 1b) without any optical aid. The latter was used in the experiments. Movement speed of the filled circle was 30 jumps/min. The duration of the test was 33 min. Reaction time, the number of correct (32 possible) and of incorrect responses were evaluated.

EEG

Subjects were separated from staff by a curtain and monitored with a video-system. EEG was recorded with an electrode cap (Electro Cap, Co., USA) [4] for 7 minutes with closed eyes, for 5 minutes with open eyes, and for 6 minutes during the Color Word Stress test (CWS) of the Swedish Performance Evaluation System (SPES) [9]. Localisation of electrodes is explained in Figure 2. Subjects were relaxed during recording EEG with closed and open eyes. 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 anyone of the colours. The task was to press a key as fast as possible when there was congruency between the meaning of the word and the colour of the text. The interval between subsequent stimuli was 1.5 seconds. Proportion of critical stimuli was 75%. Reaction time and number of errors were registered in order to control performance of subjects. Analogue 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 digitised (512 Hz/12 bit) and then transferred via an optical fibre to a computer (CATEEM®, MediSyst). The high input impedance of the amplifier (AC = 10 MΩ, DC = 20 MΩ) ensured a sufficient signal-to-noise ratio. The analogue signals were displayed on a screen for visual

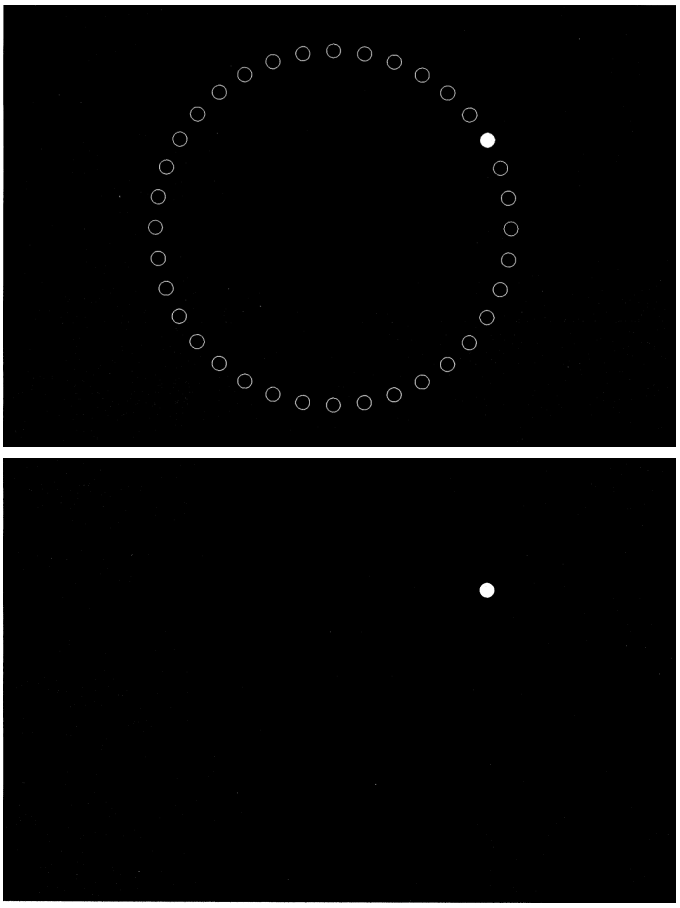

Fig. 1.(a) Mackworth clock vigilance test, version Quatember-Maly. *(b)* Mackworth clock vigilance test, version Müggenburg 33.

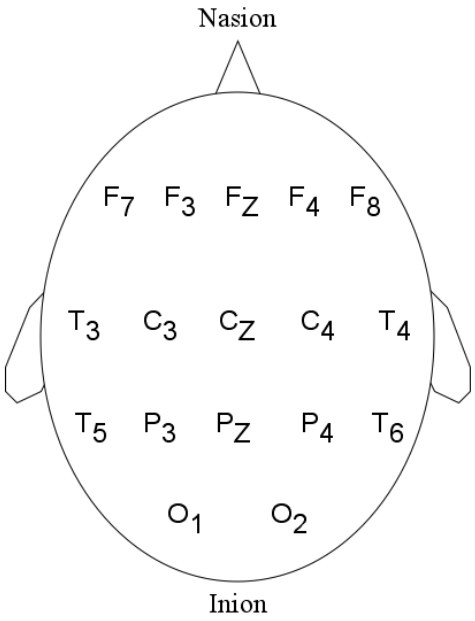

Fig. 2. Electrode scheme.

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 recognised automatically by a computer with adjustable sensitivity and 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 eyes were open. Therefore, these epochs could not be eliminated and the β_1- and β_2-band at single electrodes were not analysed. Common average reference was calculated from the signals of 16 electrodes measured against C_Z, hence obtaining signals from 17 real electrodes against a virtual reference [12]. After smoothening, the signals of 4 consecutive data points were averaged, giving an effective sampling rate of 128 Hz. Frequency analysis was performed using a Hanning window and a Fast Fourier Transformation of epochs of 4 seconds. Power spectra were calculated with a spectral discrimination of 0.25 Hz. The frequency spectra were divided in 6 frequency bands (δ = 1.25 to 4.50 Hz, θ = 4.75 to 6.75 Hz, α_1 = 7.00 to 9.50 Hz, α_2 = 9.75 to 12.50 Hz, β_1 = 12.75 to 18.50 Hz, β_2 = 18.75 to 35.00 Hz). Absolute power of the 6 frequency bands was calculated.

Visual Evoked Potentials

VEP were recorded in a dark room, using CATERPA® (MediSyst), developed from CATEEM®. 150 checker board reversal patterns of 1 Hz were displayed on a screen. The field subtended 13.1 x 9.9 degrees at a viewing distance of 129 cm. A square had 50 minutes of arc. A red point for fixation was provided in the centre of screen. The contrast was 97%. It is defined as the difference between maximum and minimum luminance divided by the sum. Artifacts were eliminated by adjustable amplitude limits of the EEG signals and of the electrooculogram. Signals were filtered with a bandpass of 0.25-280 Hz. Baseline was calculated as the mean of epochs of 100 ms duration before the stimuli. We evaluated the latencies and the amplitudes of the P100 at the electrodes O_1 and O_2. The P100 is a major positive peak at approximately 100 ms latency.

Experimental Procedure

To avoid learning effects during the experiment, subjects were acquainted with the questionnaires, simple reaction time test, vigilance test, VEP and recording of EEG during the Color Word Stress test. Subjects were instructed not to drink coffee, black tea and caffinated soft drinks before or during the experiment. Immediately before each exposure, a short medical check was performed and blood samples were taken. Blood alcohol levels of all subjects did not exceed 50 mg/l (0.05‰). Experiments were performed following a standardised protocol. Two subjects were exposed on each experimental day. The first subject entered the chamber for baseline measurements (questionnaires, EEG, simple reaction time, vigilance) at 8.05 a.m., while the chamber was supplied with filtered air. The subject was separated by a curtain from staff. After having the tests completed, he left the chamber. Then VEP were recorded in an adjacent room and reference measurements of the second subject started. After having left the chamber, solvent vapours or air were fed into the chamber. When the target concentration was reached and stable, the first subject entered the chamber (11.20 a.m.), the second followed 75 minutes later. Measurements started once again after 3.25 hours of exposure. Contact lenses were not worn during exposure. Subjects received rolls and mineral water at their request.

Statistical Analysis

All values measured during exposure were related to baseline values obtained before exposure. Explorative statistics of target variables but EEG were performed with Mann-Whitney tests after calculating differences of the post- and pre-values. Scores obtained from questionnaires were markedly skewed. Thus, analysis of variance was not possible even after logarithmic transformation. All tests were performed two-sided but the judgements of exposure and the questionnaire of SPES, as protective effects of exposure are improbable. The alternative hypothesis was that scores were higher during exposure. $p \leq 0.05$ was considered significant.

EEG-data (spectral power) were obtained from 17 electrodes in 6 freuency bands each (δ, θ, α_1, α_2, β_1, and β_2). Quotients of post- and pre-values were calculated. Medians of quotients were compared exploratively by Mann-Whitney tests. Then, multivariate analysis was performed. Logarithmic transformation revealed normal distributed data according to the criterion "skewness between –1 and +1". Thus, effect measures in the confirmative analysis refer to ratios of power spectra. An explorative analysis (results not given) showed that power values in the same band at different electrodes were highly correlated. According-

ly, for each band a separate analysis including values of all electrodes was performed. A general linear model was applied with group and electrode position as factors. Dependency of results from the same subject at different electrodes was adjusted by the method of generalized estimating equations (GEE) [13]. With this method correct standard errors and P-values are obtained for correlated data from the same subject. $p \leq 0.05$ was considered significant.

Results

Biological Monitoring

Concentration of toluene in venous blood increased mainly during the first 30 minutes, whereas acetone concentrations increased during the whole exposure (Table 1).

Ratings of Exposure

Exposed subjects rated exposure higher than the control persons (Table 2). Though not exposed, the controls rated "2" on the ordinal scale and not "1", indicating subjective feeling of exposure upon entering the chamber. After some experimental days, staff was able to differentiate between solvent exposure and sham by smell, so staff was not blinded to exposure.

Table 1. Concentrations of toluene and acetone in blood (n=12) before, during and after exposure to the mixture of toluene and acetone.

Parameter	Toluene [mg/l]		Acetone [mg/l]	
Time of exposure	mean	SD	mean	SD
Before[a]			1.19	0.29
After 30 min	0.12	0.02	2.46	0.53
After 60 min	0.13	0.03	2.91	0.78
After 105 min	0.14	0.03	3.76	1.08
After 180 min	0.14	0.03	4.35	0.81
After 270 min	0.14	0.04	5.43	1.37
30 min after end	0.11	0.03	4.68	1.13

[a]: n = 11

Table 2. Judgements of exposure of test subjects (medians (1st and 3rd quartiles)).

Parameter	Toluene/acetone	Sham	p[a]
Judgement immediately after entrance	3 (3, 3.75)	2 (1.25, 2)	<0.001
Level of confidence	4 (4, 5)	4 (4, 5)	n.d.
Judgement before leaving	2 (1, 2)	1 (1, 1.75)	0.03
Level of confidence	4 (3.25, 5)	5 (4, 5)	n.d.

[a]: One-sided Mann-Whitney test
n.d.: not determined

Table 3. Subjective symptoms, questionnaire of SPES [9] (3rd quartiles).

	Before exposure		1 min exposure		135 min exposure		End of exposure	
Item	Toluene/ acetone	Sham	Toluene/ acetone	Sham	Toluene/ acetone	Sham	Toluene/ acetone	Sham
Headache	0	0	0	0.75	0	1	1	2
Dizziness	0	0	0	0	0	0	0	0.75
Nausea	0	0	0	0	0	1	0.75	1
Tiredness	0.75	1	0.75	1	2	1	2.75	2
Pain or pressure over the chest	0	0	0	0	0	0	0	0
Coughing spells	0	0	0	0	0	0	0	0
Shortness of breath	0	0	0	0	0	0	0	0
Irritation to the eyes	0[a]	0[b]	0[a]	0[b]	0.25[a]	0.5[b]	0.25[a]	0.5[b]
Watering eyes	0[a]	0[b]	0[a]	0[b]	0.25[a]	0[b]	0[a]	0[b]
Blurred sight	0[a]	0[b]	0[a]	0[b]	0[a]	0[b]	0[a]	0[b]
Irritation to the nose	0	0	0	0	0	0	0	0
Running nose	0	0	0	0	0	0	0	0
Sensation of a bad smell	0	0	3	0	1.75**	0	1.75*	0
Irritation to the throat	0	0	0	0	0	0	0.75	0
Sensation of an unpleasant taste	0	0	0	0	0	0.75	0	0
Irritation to the skin	0	0	0	0	0	0	0	0
Feeling of fainting or vertigo	0	0	0	0	0	0	0	0

3rd quartiles are given because medians were 0 but 5 values. Median scores of tiredness were 1 and 1 for solvent exposure and sham after 135 min and 1 and 2 at the end of exposure, respectively. During solvent exposure, median score of sensation of a bad smell was 0 after one minute. The difference to sham was not significant (p = 0.06).
[a] n = 6; [b] n=9; *p <0.05; **p <0.01

Acute Symptoms

Results of the SPES questionnaire are given in Table 3. The odour of the mixture was felt significantly more unpleasant. The scores of the other items did not differ significantly. The score for tiredness in the Seeber questionnaire was slightly and significantly elevated after 135 minutes, but not at other times (Table 4). The scores for tension, complaints, and annoyance were similar in the exposed and in the control group.

Simple Reaction Time

Solvent exposed subjects performed as well as control persons. Neither reaction time nor number of hits differed significantly. Median differences of reaction time (end of exposure – before exposure) were –2 msec and 3 msec for solvent exposure and sham, respectively. Median differences of number of hits were 0 and 0.

Vigilance

Like the reaction time task, the vigilance test did not reveal any significant differences in performance. Median differences of reaction time (end of exposure – before exposure) were -25 msec and –40 msec for solvent exposure and sham, respectively. Median differences of number of correct responses were 0 and 1, those of incorrect responses 0 and –1.

EEG

In explorative univariate analyses, median spectral power decreased at all electrodes of the α_2-band in the closed eye condition. The level of significance was met at F_7. The P-value was 0.09 at two other frontal elec-

Table 4. Ratings of subjects, questionnaire of Seeber et al. [21] (medians (1st and 3rd quartiles)).

	Before exposure		1 min exposure		135 min exposure		End of exposure	
Item	Toluene/ acetone	Sham	Toluene/ acetone	Sham	Toluene/ acetone	Sham	Toluene/ acetone	Sham
Tension	0 (0, 1)	0 (0, 1)	0 (0, 0.75	0 (0, 0.75	0 (0, 1.75)	0 (0, 0.75)	1 (0, 2)	1 (0.25, 1.75)
Tiredness	0 (0, 1)	0.5 (0, 1)	0 (0, 1)	0.5 (0, 1)	1* (0.25, 2)	1 (0, 1)	1 (1, 2)	1.5 (1, 2.75)
Complaints	0 (0, 0.75)	0 (0, 0)	0 (0, 0)	0 (0, 0.75)	0 (0, 0)	0 (0, 1)	0 (0, 1)	0 (0, 1)
Annoyance	0 (0, 1)	0 (0, 1)	0 (0, 2)	0 (0, 1)	1 (0, 3)	0 (0, 1)	1 (0, 3)	1 (0.25, 1.75)

*p<0.01

Table 5. Confidence intervals of spectral EEG power, measured at 17 electrodes, during solvent exposure related to air exposure.

Band	Closed eyes	Open eyes	Color Word Stress test
δ	1.04 (0.86-1.25) 0.70	0.97 (0.82-1.16) 0.77	0.94 (0.85-1.05) 0.29
θ	0.97 (0.83-1.13) 0.69	0.88 (0.74-1.05) 0.15	0.92 (0.81-1.03) 0.14
α_1	0.97 (0.73-1.29) 0.87	1.04 (0.84-1.29) 0.71	1.03 (0.86-1.22) 0.76
α_2	0.86 (0.66-1.12) 0.24	1.04 (0.83-1.32) 0.71	1.00 (0.86-1.17) 0.78
β_1	1.0 (0.88-1.13) 0.99	0.95 (0.82-1.09)* 0.45	1.00 (0.89-1.11)* 0.79
β_2	1.01 (0.89-1.16) 0.88	1.01 (0.87-1.16)* 0.93	1.09 (0.93-1.27)* 0.28

First row: ratio of power spectra in exposed and non exposed subjects adjusted for electrode position (95% Confidence limit).
Second row: P-Value of test against no difference (ratio = 1) between groups (exposed vs. non exposed)
*: Only 12 electrodes were evaluated because of muscle artefacts.

trodes (results not shown). The main result in the EEG recorded with open eyes was a decrease of power in the θ-band except two central electrodes. Changes were not significant but at P_3. During the Color Word Stress test, power decreased in the θ-band except two central electrodes, too. Changes were significant at F_8, T_5, and P_3. Multivariate analysis did not show any significant differences between both groups for the EEG-bands for all conditions (Table 5).

Visual Evoked Potentials

P 100 latencies as well as amplitudes did not differ significantly between the solvent exposed and the control group. Median differences (end of exposure – before exposure) of latencies, measured at O_1, were –1.9 msec and 3.5 msec for solvent exposure and sham, respectively. Values obtained from O_2 were –0.7 msec and 0 msec, respectively. Median differences of amplitudes, measured at O_1, were 1.0 μV and -0.2 μV. Values obtained from O_2 were 1.0 μV and -0.3 μV.

Discussion

Toluene and acetone are typical constituents of mixtures used at workplaces. Acute exposure to the mixture composed of the two solvents was at the German threshold limit value for mixtures (TRGS 403). Both psychophysiological and neurophysiological tests did not reveal any adverse effects. However, our results cannot be completely transferred to working conditions due to obvious differences between the experimental and the occupational exposure situation. Solvent effects might be underestimated. Our subjects were in a physically rested condition, whereas occupational exposure to solvents is commonly associated with physical effort, which usually raises pulmonary ventilation and thereby results in an increased uptake of solvents compared to a subject at rest [1]. Nevertheless, it was decided against exercising under exposure of test subjects because it would have caused sustained effects on EEG itself [14]. As to sedentary subjects, little is known why there is such a huge difference between toluene blood levels in various chamber studies [19, 22, 24]. Extrapolating reported blood levels to an exposure of 25 ppm toluene, as performed in our study, measured blood toluene concentrations fall within this range. Venous acetone concentrations of our subjects were lower than those reported by Dick et al. [6], but blood acetone concentrations following controlled exposure differed considerably in different studies [6, 26], too.

Multivariate analysis failed to show significant changes in EEG spectral power following combined toluene-acetone exposure. The statistical significance of EEG comparisons depends on (1) the observed ratio between groups or conditions, (2) the observed variance, (3) the correlation between measurements in the same subject, (4) the experimental design (interindividual comparisons between groups or intraindividual comparisons within subjects), and (5) sample size. Thus, it should be noted that in the α_2-band an average reduction to 86 % was observed in exposed as compared to non exposed subjects with closed eyes, a reduction to 88 % in the θ-band with open eyes, and a reduction to 92 % in the θ-band during the Colour Word Stress test. Due to reasons noted above, P-values were 0.24, 0.15 and 0.14, respectively. Thus, the interpretation of our results focuses primarily on confidence limits instead of P-values. Observed EEG changes point to possible neurochemical effects of the toluene-acetone mixture in human central nervous system.

In a field study, an association between changing solvent concentrations and the number of slips, trips and falls among painters was observed [10]. High concentrations of organic solvents cause prenarcotic symptoms like dizziness and feeling of drunkenness. Under extreme circumstances general anaesthesia can occur [18]. The mode of action of anaesthetics has been studied thoroughly. At surgical concentrations effects of most anaesthetics are mainly mediated by inhibitory γ-aminobutyric acid ($GABA_A$) receptors [8]. In contrast, only few organic solvents have been studied, using concentrations which frequently occur at workplaces. Exposure to 80 ppm toluene inhibited dopamine receptors in rats [25]. In vitro, only very high concentrations of toluene (29 mg/l) and of 1,1,1-trichloroethane (37 mg/l) enhanced $GABA_A$-receptor mediated synaptic currents [3]. Glycine receptors were less sensitive to those solvents [3]. Acute exposure to 200 ppm 1,1,1-trichloroethane was followed by slight sedentary changes in EEG of human subjects [16]. Thus, EEG changes were compatible with a slight agonistic effect on $GABA_A$ receptors. In contrast, acute exposure to 200 ppm methanol decreased spectral power of the θ-band, pointing to an excitatory effect [15]. Recent research indicates that the θ-band of EEG is under noradrenergic control, because medetomidine, a noradrenergic and presynaptical α_2-agonist, increased θ-power in rats [7]. Thus, methanol probably enhanced noradrenergic transmission. These examples show that acute central nervous system effects of organic solvents are different. The brain is a neuronal network with numerous transmitters involved in signal transduction processes. Consequently, it appears that acute effects of mixtures cannot be predicted by the already known effects of their constituents, they rather need to be investigated for each mixture to evaluate central nervous system effects accurately.

From the point of prevention possible interactions between occupational solvent exposure with other substances like ethanol or certain medications, which bind at central nervous system receptors, might be relevant. As the toluene-acetone mixture caused insignificant EEG changes, interactions cannot be excluded. In the field study on the painters [10] mentioned above, alcohol consumption was no risk factor for near-misses. However, the authors could not exclude underreporting of alcohol ingestion. They did not mention if the effects of medications had been investigated.

In conclusion, acute exposure to a mixture consisting of toluene and acetone, not exceeding the German TLV, did not cause adverse effects on human central nervous system. However, possible subclinical effects cannot be excluded.

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Address for correspondence:
Dr. Axel Muttray
Institute of Occupational, Social and
Environmental Medicine
University of Mainz
Obere Zahlbacher Straße 67
D-55131 Mainz, Germany
Office-No: 0 61 31 – 393 32 33
Fax: 0 61 31 – 393 66 80
Email: amuttray@uni-mainz.de