Acute effects of trichloroethylene on blood concentrations and performance decrements in rats and their relevance to humans

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Abstract

This study was designed to clarify the nature of effects of trichloroethylene (TCE) on the central nervous system, and to determine the critical concentrations in blood associated with specific behavioural changes. This was achieved by a follow up of the whole time course of TCE intoxication during and after exposure. The effects of a single four hour exposure to TCE on signalled bar press shock avoidance in rats were tested by methods previously applied to investigate the acute neurobehavioural effects of exposure to toluene. The effects of TCE on the central nervous system were different from those of toluene. Even low exposure to TCE induced shock avoidance performance decrements in rats. Rats exposed to 250 ppm TCE showed a significant decrease both in the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with controls. The rats did not recover their pre-exposure performance until 140 minutes after the exhaustion of TCE vapour. Exposures in the range 250 ppm to 2000 ppm TCE for four hours produced concentration related decreases in the avoidance response rate. No apparent acceleration of the reaction time was seen during exposure to 1000 or 2000 ppm TCE. The latency to a light signal was somewhat prolonged during the exposure to 2000 to 4000 ppm TCE. It is estimated that there was depression of the central nervous system with slight performance decrements and the corresponding blood concentration was 40 µg/ml during exposure. Depression of the central nervous system with anaesthetic performance decrements was produced by a blood TCE concentration of about 100 µg/ml. These results showed effects of TCE on the central nervous system that were considered to be a function of both the exposure concen-

tration and the duration of exposure, which are closely related to the TCE concentration in blood.

(British Journal of Industrial Medicine 1993;50:470-480)

Trichloroethylene (TCE) has long had application as a degreasing agent and solvent in industry. Evidence that TCE might be a carcinogen has reduced its use during recent decades, but it is still widely used because of its powerful degreasing action. It has also been used clinically as a surgical anaesthetic and analgesic.¹

It is highly lipid soluble and readily distributes in the central nervous system. Symptoms of the central nervous system such as headache, dizziness, drowsiness, fatigue,²⁻⁴ abnormal electroencephalogram,^{5,6} and deficits in psychomotor performance⁷⁻⁹ have been reported in workers exposed to TCE. Various experimental models have been used in the study of the disorder of neurobehavioural function caused by the exposure to TCE.¹⁰⁻¹⁴ From the available information, however, it is difficult to decide such basic issues as what the extent or threshold of the effects is, and how are acute neurobehavioural effects of TCE different from those of other organic solvents. Also, there is little evidence available regarding the whole time course of TCE intoxication during and after exposure. With experimental exposures to organic solvents it is especially important to measure solvent concentrations to assess possible threshold or saturation effects or other dose related non-linearities.¹⁵

From this viewpoint, we investigated the neurobehavioural effects of a single exposure to TCE in rats by the performance of conditional avoidance response, which was the same method applied to test acute toluene neurotoxicity.¹⁵ We also estimated the critical blood TCE concentrations, which is believed to be related to the performance disorder.

Methods

ANIMALS AND APPARATUS

We used male Wistar rats seven weeks of age. All experiments were performed under a dark-light

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Figure 1 Performance changes of rats during and after a four hour inhalation exposure to 250 ppm TCE.

cycle consisting of alternating 12 hour periods, with the dark period beginning at 7 00 am. The apparatus used in the behavioural test consisted of Skinner boxes placed in a gas chamber and programming and recording equipment. A shock generator and scrambler (BRS/LVE) was used to deliver a 2.5 mA DC shock to the grid floor of each Skinner box for 0.2 s. The experimental contingencies were controlled by a Sord M23 microcomputer and related peripherals.

EXPOSURE

Static exposure was carried out in two similar stainless steel gas chambers (70 × 70 × 120 cm).¹⁶ Two chambers were simultaneously exposed, with the calculated volume of TCE introduced from an upper inlet by a vaporiser. Rats were exposed to various concentrations of TCE (250, 500, 1000, 2000, and 4000 ppm in ascending order) and to air under identical conditions in each exposure. The concentration of TCE vapour was determined with

Figure 2 Performance changes of rats during and after a four hour inhalation exposure to 500 ppm TCE.

a Shimadzu 4 BPTF gas chromatograph.

BEHAVIOURAL PROCEDURES

The shock avoidance response (fixed negative interval schedule with a light signal)¹⁷ was used to establish the behavioural baseline for the observation changes after exposure to TCE. The animals were

trained for one hour every two days with a reinforcement schedule having a 10 second shock to shock interval and a light signal was presented for five seconds before every electric shock. Under this schedule, the animals were able to avoid an electric shock if they pressed the lever when the light signal was on.

Figure 3 Performance changes of rats during and after a four hour inhalation exposure to 1000 ppm TCE.

The behavioural baseline was established in the animals after 10 to 15 training sessions. After this, eight rats in which avoidance rate succeeded in over 80% of the trials were selected and exposed first to air as a control and then to TCE and air alternatively. The animals were placed into Skinner boxes to test their lever pressing behaviour under the described avoidance schedule from 9 00 am every day. They were exposed to TCE or air for four

hours from 10 00 am to 2 00 pm and the observations were continued for about two hours after the exhaustion of TCE. The interval between exposure to TCE in any rat was set at 10–20 days to avoid lingering effects of the previous exposure. The numbers of lever presses and shocks received per 20 minute period during these seven hour test periods were adopted as the behavioural test variables.

Figure 4 Performance changes of rats during and after a four hour inhalation exposure to 2000 ppm TCE.

SOLVENT ANALYSIS

To measure the concentrations of TCE in blood during and after exposure, 140 male Wistar rats equivalent in weight to the animals of the behavioural experiment were used. They were exposed to TCE for four hours at the same concentrations as those in the behavioural experiments (250, 500, 1000, 2000, and 4000 ppm). The rats were decapitated and blood was dispensed into all

glass containers with heparin. The concentration of TCE in blood was determined by a modified gas chromatographic equilibration method.¹⁸

STATISTICAL ANALYSIS

Effects of TCE were evaluated by comparing the performance of rats during and after exposure with their own performance in air. Statistical significance was determined by three way analysis of variance in

Figure 5 Performance changes of rats during and after a four hour inhalation exposure to 4000 ppm TCE.

which the exposure effects, time effects, and the differences within eight individual rats were included as the three factors.

Results

Rats exposed to 250 ppm TCE showed a significant decline in both the total number of lever presses and in avoidance responses at 140 minutes of exposure compared with the controls. The rats did not recover to the level of performance before the exposure until 140 minutes after the exhaustion of TCE vapour (fig 1).

The 500 ppm dose of TCE induced a significant decrease in total lever presses at 120 minutes of exposure compared with the matched air controls. The avoidance response rate was significantly lower than that of controls at periods before and after 240 minutes of exposure (fig 2).

Rats exposed to 1000 ppm TCE for at least 20 minutes produced a statistically significant decrease in the avoidance response rate. Performance decrements continued during all periods of four hour exposure and even until 140 minutes after the removal of TCE vapour (fig 3).

The avoidance response rate decreased more after exposure to 2000 ppm TCE, to about 40% compared with the pretest performance. Under

these circumstances, however, the mean number of total lever presses did not change greatly during exposure periods. This was mainly because of large variability among the animals when they were exposed to 1000 ppm and 2000 ppm TCE. Two out of eight rats showed anaesthetic performance decrements when exposed to 1000 and 2000 ppm TCE, whereas two other rats showed responses somewhat higher than the baseline level under the same exposure conditions (fig 4).

Both the number of lever presses and the avoidance response rate decreased drastically after exposure to 4000 ppm TCE. Anaesthetic performance disorders such as ataxia or no response to either light or electric shock were found in all exposed rats. After stopping exposure, one rat died, and seven of the eight rats showed a gradual recovery of performance (fig 5).

Figure 6 summarises the dose-response relation of the number of avoidance responses under the different concentrations of TCE. The number of avoidance responses decreased under all of the concentrations studied. Exposure to 250 to 2000 ppm TCE produced a concentration related decrease in the avoidance response, whereas 4000 ppm TCE had rather different features. Avoidance performance under 4000 ppm TCE was closely related to

Figure 6 Dose-response relation of TCE exposure to the avoidance responses. Each point indicates the mean of the avoidance responses expressed as a percentage of the pretest of eight rats during seven hour test periods under each TCE concentration. The dotted shadows show the range of the control exposure (air), which includes the maximum and minimum points.

Figure 7 Dose-response relation of TCE exposure to latency. Each point indicates the mean of the time elapsed from the start of a light signal to the lever press response. The dotted shadows show the mean (SD) of the control exposure (air).

the time during and after exposure to TCE.

Figure 7 shows the latency, that is, the time elapsed from when the light was delivered as a signal in the conditioned avoidance response until the time when the rat pressed the lever. The latency to the light signal was prolonged during the exposure to 1000 to 2000 ppm TCE. Acceleration of the reaction time was only seen before the anaesthetic phase at the beginning of exposure to 4000 ppm.

Figure 8 shows TCE uptake (inhalation) and elimination in the blood of rats exposed to TCE concentrations of from 250 to 4000 ppm for four hours. As a whole, there was a progressive increase in the quantity of TCE in blood throughout the

four hour inhalation period. Most of the increase occurred during the initial hour of exposure. The time required for equilibrium in blood to be reached was about 40 minutes. The concentration of TCE in the blood after a single four hour exposure was linearly dependent on the concentration of TCE inhaled by the animals exposed to 250 ppm to 2000 ppm TCE (fig 9). By contrast, the concentrations of TCE in blood of rats exposed to 4000 ppm TCE were lower than expected. The anaesthetic effects seemed to cause the suppression of the pulmonary uptake of TCE under 4000 ppm exposure.

Figure 10 shows the relation between the avoidance response rate and the blood concentration,

Figure 8 Concentration of TCE in blood of rats during and after a four hour inhalation exposure to TCE. Each point represents the geometrical mean for six rats.

estimated from the result of each exposure. Shock avoidance performance decrements occurred when the TCE concentrations were from 10–40 µg/ml of blood and central nervous system depression with anaesthetic performance disorders was produced by blood TCE concentrations of over 100 µg/ml. The mean performance decrements when exposed to 1000 or 2000 ppm TCE seemed to be a biphasic and non-linear function of the blood concentration, mainly because of the great variability of response of individual rats under both TCE exposures, as already mentioned.

Discussion

The data reported on the neurobehavioural effects of low exposure to TCE are conflicting. An experiment on humans⁹ in which volunteers were exposed for two four hour exposures of 110 ppm TCE indicated significant alterations in performance on tests of tachistoscopic perception, immediate memory, and complex reaction time (both in response latency and regularity), whereas several subsequent attempts to obtain behavioural effects at concentrations of between 100 and 300 ppm have failed.^{19–22}

Exposure to TCE up to 200 ppm is commonly thought to cause only subtle or no effects on the behaviour of experimental animals in acute exposures.^{10–12} When rats were trained to swim a 4 m alley with and without a 27 g load attached to their tails, an 800 ppm concentration of TCE increased

swimming time, but only with the load. Activity measured in other groups of rats showed that only exposure to 1600 ppm TCE for five hours resulted in decrements in motor activity.¹³ Our experiment, however, indicated that even a low exposure to TCE such as 250 ppm for four hours resulted in shock avoidance performance decrements in rats (periods D and E, fig 1). This coincides well with a human exposure study with TCE,³ which showed that during exposure to 200 ppm TCE for seven hours, 80% of the subjects reported fatigue and sleepiness and 50% reported that it took greater mental effort to perform the Romberg test.

Exposure to ranges of 250 ppm to 2000 ppm TCE for a period of four hours produced concentration related decreases in the avoidance response; nevertheless, the number of lever presses did not change and no excitatory behaviour was seen. By contrast, in our study on toluene¹⁵ using the same behavioural and toxicological techniques, concentration related increases in lever presses and acceleration of the reaction time were found, which meant that toluene was, at concentrations such as 1000 or 2000 ppm, excitatory, although at other concentrations such as 4000 ppm, it was depressive. Both TCE and toluene have been abused because of the euphoric effects that some people experience on inhaling vapours—for example in

Figure 9 Blood TCE concentration in rats exposed to TCE just after a four hour inhalation. Each point represents the geometrical mean (2 SEM).

Figure 10 Relation between number of avoidances and blood TCE concentration. The mean (SEM) of lever pressing in eight rats at 20–40 minutes, 100–120 minutes, and 220–240 minutes after exposure are plotted against the mean TCE concentrations in the blood measured at the corresponding time.

one study about 4% of the workers were described as abusers.²³ From our present experiment, however, it was clearly indicated that the acute effects of TCE on the central nervous system were quite different from those of toluene.

Special attention should be paid to the fact that the performance disorders continued and sometimes were more clear after the cessation of exposure than during exposure. The pronounced performance decrement as a result of exposure to 250 ppm or 500 ppm TCE continued for 80 to 140 minutes after the end of exposure (periods F, G, and H, figs 1 and 2). Why performance decrements after the termination of exposure to 250 ppm and 500 ppm TCE were significant might be because of the metabolite of TCE.^{24–26} As has been shown by many studies one third to one half of the retained amount of TCE is metabolised to trichloroethanol and excreted in the urine as urochloral acid. Trichloroethanol seems to play an important part in the pharmacodynamic and toxic effect of TCE—

for instance, it was reported that trichloroethanol was at least three, and probably five to six times more effective at the same dose as TCE as measured by the threshold current intensity of electrical skin stimulation and the electrical excitability of the motor cerebral cortex.²⁷

There are only a few reports with data on blood concentrations of TCE during and after exposure. Astrand and Ovrum²⁸ reported that during exposure to 540 mg/m³ (100 ppm) and 1080 mg/m³ (200 ppm) of TCE, the arterial blood concentration increased linearly with the concentration in the alveolar air. The blood concentrations of TCE at rest after 30 minutes of exposure were 1.1 mg/kg and 2.1 mg/kg, respectively and during a 50 Watt exercise, 2.7 mg/kg and 6.0 mg/kg respectively. Sato *et al*²⁹ reported that just after the termination of a four hour exposure to 100 ppm TCE, blood concentration was 170 µg/100 ml of blood. From these experiments on humans and our animal study, there seem not to be such large differences of

concentration of TCE in blood between rats and humans. This is congruent with the fact that standardised metabolic capacities in rats and humans were considered to be similar to each other as judged by physiologically based pharmacokinetic data on trichloroethylene.³⁰

The major purpose of this study was to investigate the critical concentration in blood associated with specific behavioural changes during and after exposure to TCE to understand the possible threshold and dose-response relation. According to our results, the effects on the central nervous system seem to be functions of both the exposure concentration and its duration, and are related to the increases in concentration of TCE in blood. It is estimated that depression of the central nervous system with slight performance decrements occurred when the blood concentration was 10 µg/ml during exposure. Depression of the central nervous system with anaesthetic performance decrements was produced by a blood concentration of TCE of about 120 µg/ml. Blood samples can be considered to be a reasonable index of the behavioural effects of TCE in experimental subjects, although the brain has a somewhat higher tissue-blood partition coefficient for solvents than the liver and most other tissues except fat.³¹

In a physiological study with squid axons in vitro,³² TCE decreased axonal action potentials in proportion to the solvent concentration. The potentials returned to the central range after the solvent was removed from the medium. It is also reported that the acute depressive effects of TCE are due to effects on the excitable neural membranes.³³ Behavioural effects found in the present study on exposure to TCE may be due to this physiological background.

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Accepted 6 July 1992