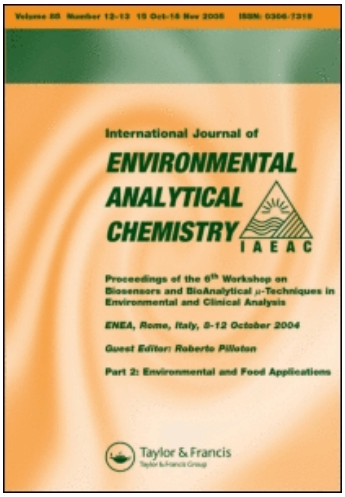

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The Use of Automated Headspace Gas Chromatography for Determination of 1,1,1-Trichloroethane in Rat Blood and Brain Tissue

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1,1,1-trichloroethane in blood and brain tissue from rats which had been artificially ventilated with solvent (8000 ppm) was analysed by automated headspace gas chromatography using a fused silica capillary column. A given concentration of 1,1,1-trichloroethane in the brain could be correlated with a corresponding concentration in the blood; both the uptake and release of the solvent were quicker in blood than in brain. No volatile metabolites of the solvent were found. Automated headspace gas chromatographic analysis is a rapid and sensitive technique for the quantitative registration of volatile organic solvents, e.g. of industrial importance, in body fluids and tissues.

KEY WORDS: 1,1,1-trichloroethane, headspace gas chromatography, blood, tissue.

INTRODUCTION

1,1,1-trichloroethane (methylchloroform) is an organic solvent with widespread industrial use, mainly for de-greasing purposes. Chlorinated hydrocarbons are known to cause acute and chronic damage to the nervous system. It would, therefore, be highly desirable to be able to correlate findings of circulatory, metabolic or structural damage to the brain after exposure to such a solvent with the concentration of the same solvent, for example, in the blood.

The aim of the present study was to investigate the time course of the uptake and elimination of 1,1,1-trichloroethane by rats which had been artificially ventilated with a high concentration of solvent in the insufflated

gas mixture. Gas chromatographic headspace analysis was used for evaluation of solvent concentrations in blood samples and brain tissues.

METHODS

Animals and operative techniques

The experiments were performed on male rats (S.P.F. Wistar strain, Møllegaard Avlslaboratorium, Copenhagen, Denmark) weighing 260–435 g. The animals were fed on pellet diet (Astra–Ewos) and had free access to tap water. Anesthesia was induced with 3% halothane. The animals were then tracheotomized, immobilized by intravenous injection of tubocurarine chloride (0.5 mg/kg) and ventilated with a Starling type respirator, which delivered a gas mixture of N_2O and O_2 (3:1, v/v) containing 0.7% halothane. Ventilation was adjusted to yield an arterial PCO_2 of 35–40 mmHg. Body temperature was kept at 37°C. One femoral artery was cannulated to be used both for blood pressure recording with an electromanometer and for sampling of blood. A skin incision was made over the skull bone to accommodate a plastic funnel for later freezing of the brain *in situ* with liquid nitrogen.[1]

After the operative procedures had been completed, the animals were given heparin intravenously and maintained on N_2O and O_2 (at the same concentrations as above) for 30 to 60 minutes in order to eliminate halothane before the exposure to 1,1,1-trichlorethane was started.

All rats were exposed to 8000 ppm (43.7 mg/l) of 1,1,1-trichloroethane (Uddeholm, Genklene LV, ICI, Runcorn, England)—a dose making an awake animal atactic—in the N_2O/O_2 atmosphere. The solvent concentration of the inspired gas was repeatedly measured by gas chromatography. The animals were kept exposed to 1,1,1-trichloroethane for one or two hours after which they were killed by freezing the brain with liquid nitrogen—either directly, or after the solvent had been turned off and the rats been ventilated with N_2O/O_2 for another one or two hours.

Sampling

Arterial blood samples were anaerobically taken from the catheter in glass capillaries before, during and after the exposure period. The capillaries were connected to a calibrated syringe, which delivered a constant volume (75 or 150 μl) of blood into the glass vials used for headspace analysis (see below). The vials were immediately sealed with an aluminium cap around a Teflon lined rubber septum. Prior to headspace gas chromatography, 5 μl (3.85 mg) of hexadecane (Merck, Schuchardt, W. Germany), used as an

internal standard (or compensation standard, see Kolb[2]) was added to the vials by puncturing the rubber membrane with a syringe.

The brains were chiselled out during irrigation with liquid nitrogen and kept at −80°C until analysis. The samples were ground in liquid nitrogen in order to prevent solvent evaporation. Portions of pulverized tissue (200–500 mg) were transferred to the vials which were immediately sealed. Hexadecane (internal standard) was added and the samples were subjected to headspace analysis (see below).

Quantitation

Standard solutions containing varying amounts (0.015–0.1%, v/v) of 1,1,1-trichloroethane in decanol were analysed by injecting 1 μl portions of the liquid samples onto the column (see below). In addition, standards (5 μl) of a solution of 1,1,1-trichloroethane (6.7–670 μg) in hexadecane and added to blood samples and brain tissues taken before solvent onset, were analysed by headspace gas chromatography. These two series of experiments enabled the areas of peaks representing 1,1,1-trichloroethane in the head-space analyses to be correlated with a corresponding actual concentration in the blood and brain.

Gas chromatography

A dual-channel gas chromatograph (Perkin–Elmer F-45) was used for automatic headspace analysis. The glass vials were heated at 75°C for at least 30 min in the automatic turntable of the instrument prior to the chromatographic analyses, which were carried out using split injection on a 25 m fused silica capillary column coated with SP 1000. The column temperature was 115°C. The injection period was 7 s and the carrier gas flow rate was 1.5 ml/min through the column. The attenuation of the flame ionization detector signal was held constant at 2. The analyses were carried out automatically, allowing a 12 minute chromatography period for each sample.

The standard solutions of 1,1,1-trichloroethane in decanol were analysed by injection of the liquid sample (1 μl) into the same column, connected to the second injection port of the gas chromatograph. The chromatographic conditions (including split ratio) were the same as for the headspace analyses as given above.

RESULTS

Gas chromatography

The headspace gas chromatography technique used enabled the solvent and the internal standard—hexadecane—to be separated within 6 min of

retention time. (Figure 1). The detection limit for 1,1,1-trichloroethane at the prevailing split ratio was estimated to approximately 0.5 μg, which is equivalent to 6.4 μg/g blood in a 75 μl blood sample or 2.5 μg/g brain tissue in a 200 mg tissue sample.

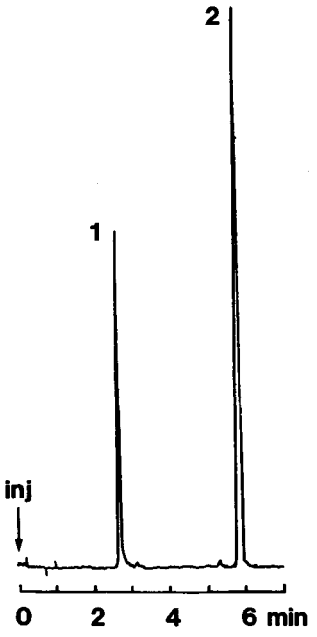

FIGURE 1 Registration by head-space gas chromatography of 1,1,1-trichloroethane in a 75 μl rat blood sample, taken after a 5 min exposure period to 8000 ppm of solvent in the inspired air. See text for specified preparatory and analytical test conditions. The peaks represent 1,1,1-trichloroethane (1) and hexadecane (2); the latter used as internal standard.

Uptake and release of 1,1,1-trichloroethane in blood

After 1 h of solvent exposure, the concentration in the blood reached a value of 115 μg/g blood. The concentration was rapidly decreased following ventilation with N_2O-O_2 (Figure 2). With a 2 h solvent exposure period, the highest concentration obtained was 268 μg/g blood. This concentration decreased to 27 μg/g blood after 1 h ventilation with N_2O/O_2, with a rate similar to that observed in the 1 h exposure group.

Uptake and release of 1,1,1-trichloroethane in brain

After a 2 h exposure period, the concentration of solvent in the brain tissue was about equal to the concentration in the blood (C brain/C blood = 0.9). However, the release from brain tissue was considerably slower than from blood. For example, the tissue concentrations was 2.5 times the blood concentration after 1 h ventilation with N_2O/O_2 (Figure 3).

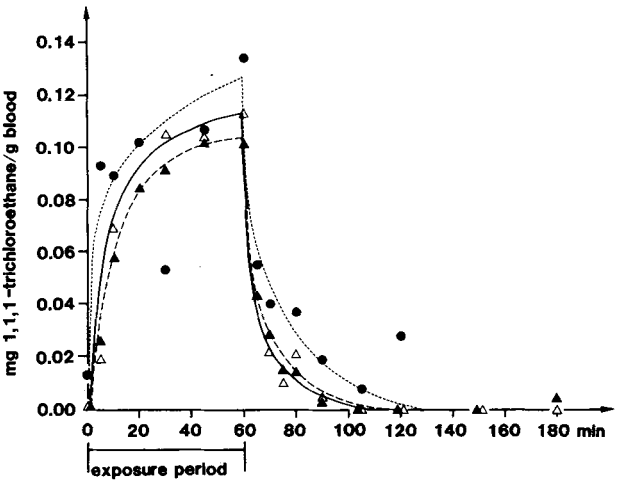

FIGURE 2 Uptake and elimination of 1,1,1-trichloroethane by three different rats artificially ventilated on N_2O/O_2 (3:1). During the first hour, the solvent was added to the inspired gas mixture (8000 ppm).

DISCUSSION

It is known that 1,1,1-trichloroethane is biotransformed to a very low extent[3,4] with almost all of the inspired solvent being expired again, unmetabolized. However, trichloroethanol and, to a lesser extent, trichloroacetic acid have been found in urine from rats exposed to 1,1,1-trichloroethane.[5] We were unable to detect trichloroethanol or any other metabolites in the blood or brain samples. With the high resolution gas chromatographic system used trichloroethanol would have been easily discovered since it was found to be well separated from 1,1,1-trichloroethane (relative retention 3.0).

The uptake and release of 1,1,1-trichloroethane of spontaneously breathing awake rats has been studied by manual syringe injection into a gas chromatograph of head space gaseous vapours from closed vials containing tissue or blood samples homogenized in ethanol.[4] The present

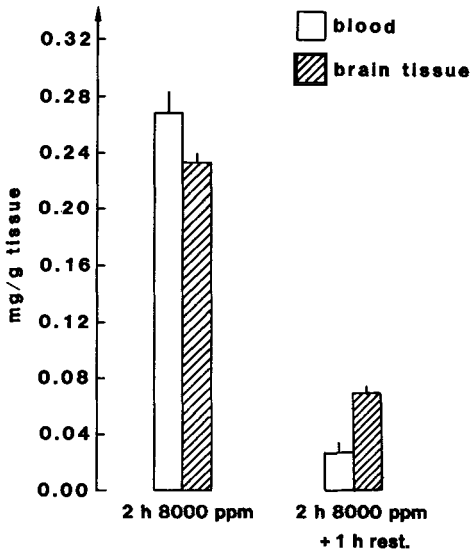

FIGURE 3 Solvent concentrations in rat blood and brain tissue after 2 h exposure to 8000 ppm 1,1,1-trichloroethane (left), and after such an exposure followed by a 1 h elimination period using pure N_2O/O_2 (right). The values are given as means of four different experiments (standard error of the means indicated).

results obtained by automated head-space gas chromatography tally with these results.

The uptake of solvent into the body is dependent on the concentration of the solvent in the inspired air. However, pulmonary ventilation, blood flow and the solubility of the solvent in blood and tissue are factors which influence the uptake.[6] It is therefore necessary not only to measure the concentration in ambient air, but also in blood samples to estimate the hazardousness of the worksite. The present study indicates that a given solvent concentration in blood can be correlated with a corresponding concentration in the brain, when studying acute exposure with relatively high concentrations, which was the purpose of the present study. Whether this is true also for lower concentrations was not evaluated. Such studies would preferably include electron capture detection, by which means the sensitivity for detection of halogen-containing solvents can be increased considerably. By contrast, the flame ionization detector used in this study is applicable for analysis of organic solvents in general. Automated head space gas chromatography constitutes a rapid and convenient means for the determination of organic solvents in blood with minimal blood volume and sample handling required.

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