Carboxyhemoglobin Elevation after Exposure to Dichloromethane

Abstract. *Inhalation of dichloromethane vapor in concentrations of 500 to 1000 parts per million for 1 to 2 hours promptly initiated the formation of significant quantities of carbon monoxide in human subjects. The evidence suggests that carbon monoxide may be a metabolite of dichloromethane and that exposure to concentrations of dichloromethane below the industrial threshold limit values may result in the formation of carbon monoxide in amounts that exceed the allowable limit.*

The inhalation of CH_2Cl_2, a solvent commonly encountered in household aerosols and widely used in industry, has been found to result in the formation of CO in man. Of the more than 4×10^8 pounds (1.8×10^8 kg) of CH_2Cl_2 produced in the United States each year, one-sixth is used in retail paint removers. Thus, a large number of persons in the nonindustrial environment, including the elderly and those with significant cardiovascular disease, may be subjected unknowingly to the anoxic effects of CO.

In an effort to evaluate the toxic potential of CO from this source, several persons were exposed to known concentrations of CH_2Cl_2 (spectrographic grade with a purity of 99.5+ percent) in a controlled-environment chamber (*1*). The air temperature in the chamber was 23°C with a relative humidity of 50 percent. These exposures were designed to simulate the type of vapor exposure encountered during the use of a paint remover as well as that commonly encountered industrially.

Eleven healthy male graduate students and medical school faculty members, ranging in age from 23 to 43 years, volunteered for the exposure studies. Each was given a comprehensive medical examination prior to his selection and 1 hour before each exposure. Prior to exposure the following tests were conducted: for the subjects in experiments 1 through 4 a complete blood count, a serum bilirubin determination, and a carboxyhemoglobin (COHb) determination were carried out on a venous blood sample; a visual evoked response (VER) was measured for the subjects in experiments 2 and 3; an analysis of an alveolar breath sample for CO (*2*) and CH_2Cl_2 (*3*) was carried out for subjects in experiments 1 through 4.

During the exposures the subjective and objective responses of each individual were recorded immediately upon entering the chamber and every 15 minutes thereafter. Every 30 minutes during exposure a venous blood sample was obtained for the determination of COHb. In experiments 2 and 3, a VER was obtained on each subject after both 1 and 2 hours of exposure.

After each exposure, venous blood samples were obtained periodically for the COHb determination and complete blood counts. Alveolar breath samples were collected in breath pipettes and 6-liter saran bags by means of the 20-second breath-holding technique for CH_2Cl_2 and CO analysis by both gas chromatographic and infrared methods (*1–3*). Urine samples collected over a 24-hour period after exposure were analyzed for urobilinogen content.

The concentration of CH_2Cl_2 in the chamber atmosphere was recorded continuously by an infrared spectrometer and periodically by a gas chromatograph (*1*). Calibration standards of CO and CH_2Cl_2 were prepared in saran bags and were analyzed by both the infrared and gas chromatographic methods before and every hour during each experiment.

In experiment 1, subject 1 was exposed for 1 hour to CH_2Cl_2 at a mean concentration of 213 ± 10.4 parts per million (ppm) (mean $\pm$ 1 standard deviation). This subject's COHb saturation prior to exposure was 0.4 percent (the normal carboxyhemoglobin content of nonsmokers is between 0.4 and 1.5 percent hemoglobin saturated with CO). During the exposure the COHb saturation rose promptly to 1.5 percent after 30 minutes and to 1.75 percent after 60 minutes. The COHb saturation continued to rise after the exposure period, peaking at a value of 2.4 percent 3 hours after the end of the exposure. This value slowly decreased to 1.5 percent 20 hours after

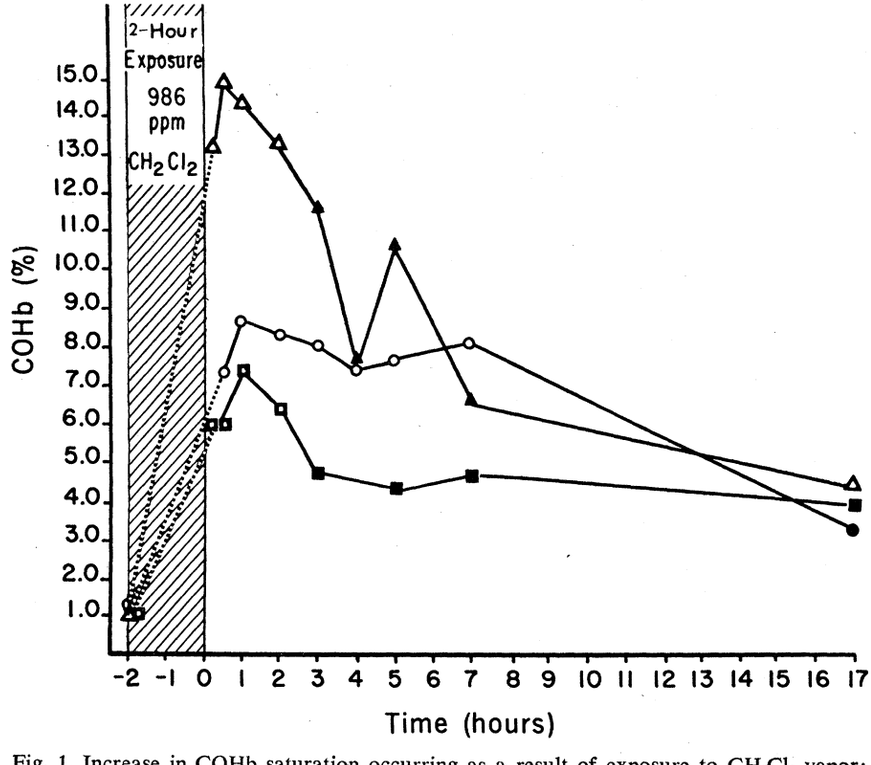

Fig. 1. Increase in COHb saturation occurring as a result of exposure to CH_2Cl_2 vapor: (open symbols) CO-oximeter COHb determinations; (filled symbols) COHb values calculated from alveolar breath data. Note the wide range in COHb saturation present in three individuals identically exposed.

exposure. The subject's hematocrit remained stable, and 24 hours after exposure all of the hematological and clinical chemistry values were normal. No untoward subjective symptoms or objective signs of illness were noted during the exposure period or during the 24-hour period after exposure.

In experiment 2, subjects 1, 2, and 3 were exposed for a 2-hour period to CH_2Cl_2 at a concentration of 986 ± 104 ppm. The three subjects reported the odor of the solvent at about 1000 ppm to be moderately strong, but not particularly objectionable. No eye, nose, or throat irritation was reported. After 1 hour of exposure, two of the subjects reported mild light-headedness which persisted throughout the remainder of the exposure period and cleared within 5 minutes after cessation of the exposure. Exposure to this concentration of CH_2Cl_2 produced alterations in the VER of all three subjects. Details of these findings will be reported elsewhere (*4*).

The COHb saturation increased in all three subjects, rising to a mean value of 10 percent 1 hour after exposure (Fig. 1). Seventeen hours after the exposure period the COHb saturation was still elevated (3.9 percent). During the 24-hour period after exposure the hematocrit for all three subjects remained normal; there was no increase in urinary urobilinogen formation, and all of the clinical chemistry and hematological values were within normal limits.

In experiment 3, subjects 1, 2, and 3 were exposed to two different concentrations of the solvent: 514 ± 9.5 ppm for 1 hour and then 869 ± 12.1 ppm for the second hour. No untoward subjective symptoms occurred during the first hour of exposure. However, within 15 minutes after the vapor concentration was increased to 868 ± 12.1 ppm one of the subjects developed definite light-headedness which persisted throughout the exposure period and for 5 minutes after the exposure period. The VER of all subjects was altered (*4*).

The base-line mean COHb saturation for the subjects prior to exposure was 0.6 percent. The COHb saturation increased continuously during the exposure period and continued to increase during the first few hours after exposure, reaching peak saturations of 8.5, 6.0, and 4.3 percent in the three subjects. The COHb saturation slowly declined after the exposure period but was still elevated above the control value 24 hours after exposure.

During the 24-hour period after exposure, all hematological and clinical chemistry values were within normal limits, and the total urinary urobilinogen excretion during this 24-hour period remained normal.

In experiment 4, subjects 4 through 11 were exposed to CH_2Cl_2 at a concentration of 514 ± 13.9 ppm for 1 hour. During this interval no untoward subjective symptoms or objective signs of illness were noted.

The base-line mean COHb saturation for the group prior to exposure was 1.5 percent. This value rose during the course of the exposure to a mean saturation of 2.6 percent. This increase in COHb saturation continued to 3.4 percent 1 hour after exposure. At 21 hours after exposure the COHb saturation was still slightly elevated, and repeat hematological and clinical chemistry studies at that time revealed no evidence of any increase in red blood cell destruction.

These experiments revealed that the exposure to CH_2Cl_2 vapor in concentrations of 500 to 1000 ppm for 1 to 2 hours promptly initiated the formation of CO in all 11 subjects tested, a result which suggests that this effect may be of universal occurrence in man. Since there was no overt evidence of increased red blood cell destruction, it would appear that the CH_2Cl_2 may be the source of the CO.

Exposure to CH_2Cl_2 vapor for 2 hours in concentrations simulating those encountered during home paint-stripping operations resulted in COHb saturations in excess of those permitted in industry from exposure to CO alone (*5*). Not only was the peak COHb saturation exceeded (the equilibrium COHb saturation after exposure to 50 ppm of CO is 7.9 percent) but the biological half-life of CO, which is approximately 5 hours in a sedentary individual (*6*), was greatly prolonged, thus intensifying the CO exposure.

On the basis of these limited data, it is impossible to predict the COHb saturation that might be reached as a result of repeated 8-hour exposures to the industrial threshold limit value (TLV) of 500 ppm for CH_2Cl_2. The COHb saturation may well be excessive, and we are of the opinion that additional investigations into the effects of CH_2Cl_2 on human beings are necessary so that the TLV for CH_2Cl_2 can be reassessed and, if necessary, readjusted so as to provide an adequate margin of safety.

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Electrical Field-Flow Fractionation of Proteins

Abstract. *Protein separation has been achieved by electrical field-flow fractionation, a heretofore unrealized separation technique. Some advantages of this method relative to electrophoresis are the low voltage required, the lack of adverse heating and support effects, and the existence of the method as an elution technique. A comparison of theoretical and experimental retention shows good agreement.*

Field-flow fractionation (FFF) is a separation method in which various applied fields, working in conjunction with cross-sectional flow nonuniformities in a narrow tube, cause the differential migration of molecules and ions (*1*). In electrical field-flow fractionation (EFFF) the applied field is electrical, making this technique applicable in theory to ionic species, particularly charged macromolecules. Here we report the first realization of EFFF separation.

The conceptual basis of EFFF is shown in Fig. 1 (*1, 2*). Laminar flow in a narrow tube establishes a parabolic flow profile having characteristic veloc-