FUNDAMENTAL AND APPLIED TOXICOLOGY 6, 162–171 (1986)

The Effect of an Unusual Workshift on Chemical Toxicity

I. Studies on the Exposure of Rats and Mice to Dichloromethane[1]

YOUNG C. KIM AND GARY P. CARLSON[2]

Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

The Effect of an Unusual Workshift on Chemical Toxicity. I. Studies on the Exposure of Rats and Mice to Dichloromethane. KIM, Y. C., AND CARLSON, G. P. (1986). *Fundam. Appl. Toxicol.* **6**, 162–171. The 10- or 12-hr workday has become increasingly popular in industry. Current occupational exposure limits are designed to protect workers on the standard 8-hr/day workshift and are not intended for use for longer workshifts. Experiments were conducted to compare the effects of a 12-hr exposure schedule to those of an 8-hr schedule on the carboxyhemoglobin (COHb) formation resulting from dichloromethane (DCM) inhalation. Rats and mice were exposed to 200, 500, or 1000 ppm DCM for 8 hr/day for 5 days or 12 hr/day for 4 days. The effect of the unusual exposure schedule on COHb levels was not significant. The metabolic pathway for the formation of COHb appeared to be saturated even at the lowest concentration of DCM. To examine the possible increase in the retention of inhaled DCM in the longer exposure schedule, single exposures for 8 and 12 hr were compared. The peak blood DCM level was dependent upon the DCM exposure concentration, but the half-life was independent of the duration of exposure and the concentration of DCM. The half-life of COHb in blood was prolonged by increasing the DCM concentration, but was not affected by the exposure period. Pyrazole treatment decreased COHb level and increased blood DCM level in rats exposed to DCM. These results suggest that the exposure limit for a chemical with a short biological half-life and readily reversible toxic effect may not need to be adjusted for a longer workshift which is in agreement with some of the mathematical models based upon the pharmacokinetics of a toxicant.

Over the past two decades a number of industries which require continuous process operations have adopted unusual or novel workshifts often involving a 3- or 4-day workweek of 12 hr/day (Brief and Scala, 1975; Wilson and Rose, 1978). Concern has been expressed that an unusual workshift may place workers at greater risk to the toxicity of chemicals because of both the increase in the exposure time and the decrease in the recovery period.

Several mathematical models have been proposed for modifying the Threshold Limit Values (TLVs), which normally assume the standard workshift of 8 hr/day or 40 hr/week (ACGIH, 1984), to provide "equal protection" for the workers on an unusual workshift. Brief and Scala (1975) addressed this problem simply by suggesting a TLV reduction factor which was based solely on the work hours and the recovery period per day. This approach was taken by OSHA (1979) on the assumption that if the total uptake of a substance were the same during a certain period, equal protection would be provided for two different workshifts. Others have attempted to incorporate factors related to the biological half-life of a chemical (Mason and Dershin, 1976; Hickey and Reist, 1977; Roach, 1978; Veng-Pedersen, 1984). Using these latter models, one can predict the uptake and elimination of a toxicant with known pharmacokinetic parameters, and the goal is to limit the body burden of the sub-

[1] Presented in part at the 23rd Annual Meeting of the Society of Toxicology, Atlanta, Georgia, 1984.

[2] To whom requests for reprints should be addressed.

0272-0590/86 $3.00

stance in a prolonged workshift to that of the standard workshift by decreasing the current exposure limit.

Very little effort has been made to investigate experimentally whether such changes in exposure schedule can alter the risk of toxicity of a chemical except for studies on carbon tetrachloride (CCl_4) (Paustenbach, 1982). These studies indicated that the 12-hr exposure of rats to 100 ppm CCl_4 for 3 or 4 days in a week for two consecutive weeks produced an increase in the residual tissue burden in the fat and lung compared to the rats exposed for 8 hr/day for 5 days each week. Prolongation of half-lives of CCl_4 in the breath, urine, and feces in the 12-hr exposure group was also observed. The 12-hr exposure group excreted a greater percentage of the CCl_4 via feces and less by exhalation when compared to the 8-hr group.

In the present studies, the effect of altering the exposure schedule from 8 hr/day for 5 days to 12 hr/day for 4 days was examined using dichloromethane (DCM) as the agent for inhalation. Dichloromethane is an important constituent of paint removers, and is widely used in industry as a degreaser, solvent, and extraction medium. Dichloromethane is considered to be relatively nontoxic except at extremely high exposure levels which may cause narcosis (Von Oettingen, 1964) and liver and kidney injuries (Heppel *et al.*, 1944; Klaassen and Plaa, 1966; Plaa, 1973).

The major toxicological hazard from exposure to low concentrations of DCM lies in the formation of carbon monoxide (CO) during its biotransformation. Stewart and coworkers (1972a,b) first reported that humans exposed to up to 986 ppm DCM for 1 or 2 hr had elevated carboxyhemoglobin (COHb) levels which were dose dependent and peaked hours after the cessation of exposure. Ratney *et al.* (1974) examined COHb levels in workers exposed to 180 to 200 ppm DCM. At the beginning of a workday, the COHb level was 4.5%, rose to 9% by the end of 8 hr and returned to 4.5% by the next morning. The estimated half-life for COHb decline was 2.5 times what would be expected if CO itself were inhaled. The extent to which COHb levels could be elevated was shown by Langehennig *et al.* (1976) who observed COHb levels of 30 to 40% obtained during furniture stripping.

The metabolism of DCM has been studied extensively by Anders and his associates (Kubic *et al.*, 1974; Kubic and Anders, 1975, 1978; Ahmed and Anders, 1976, 1978). These studies indicate that DCM is primarily metabolized to CO and CO_2. Metabolism to CO is mediated by the microsomal fraction of the rat liver requiring NADPH and oxygen. The COHb formation caused by the metabolic conversion of DCM to CO has been reported to be saturable in both humans (McKenna *et al.*, 1980) and rats (Hogan *et al.*, 1976; McKenna and Zempel, 1981; McKenna *et al.*, 1982).

In the present studies, the effect of the 12 hr/day for 4 days exposure schedule on COHb formation was examined in rats and mice. In order to measure the kinetics of DCM and COHb disappearance, a single exposure for 8 or 12 hr was also employed. Since it was reported that the rate of COHb decline in rats was faster than that of humans (Peterson and Stewart, 1970; Ratney *et al.*, 1974; McKenna *et al.*, 1982), attempts were made to decrease the rate of DCM biotransformation using a drug metabolism inhibitor.

MATERIALS AND METHODS

Animals and treatments. Male adult Swiss–Webster mice (20–25 g) (Laboratory Supply Co., Indianapolis, Ind.) and male Sprague–Dawley rats (200–250 g) (Laboratory Supply Co., or Harlan Sprague–Dawley, Inc., Indianapolis, Ind.) were used in this study. Animals were acclimated in environmentally controlled rooms (light: 0800–2000, dark: 2000–0800) for at least 4 days prior to experimentation. Lab chow (Wayne Lab-Blox, Allied Feed Mills, Chicago, Ill.) and tap water were allowed *ad libitum*. In some experiments, rats were treated with pyrazole (300 mg/kg, ip) before a single exposure to DCM for 8 or 12 hr. The daily dose of pyrazole was reduced to 150 mg/kg in the experiments with repeated DCM exposure for 4 or 5 days.

Chemicals. Drugs and chemicals used in this study include dichloromethane (Mallinckrodt, Inc., Paris, Ky.), tris(hydroxymethyl)aminomethane and pyrazole (Sigma Chemical Co., St. Louis, Mo.), sodium dithionite (Fisher

Scientific Co., Fair Lawn, N.J.), and heparin (Abbott Lab., North Chicago, Ill.).

Inhalation exposures. Exposures to DCM were conducted in a 30-liter cylindrical glass chamber maintained under dynamic air-flow conditions. The total air flow was 16 liters/min. The DCM concentrations in the chamber were achieved by passing air through a gas-washing bottle containing DCM. The chamber concentration was measured hourly using a Varian Model 3700 gas chromatograph (GC; Varian Instrument Division, Palo Alto, Calif.) equipped with a flame ionization detector or monitored continuously using a MIRAN 1A gas analyzer (Foxboro Analytical, South Norwalk, Conn.). For gas chromatography, the chamber air was sampled and injected into the GC. The column was packed with 5% OV-17 on 80/100 Chromosorb G-HP (Anspec Co., Ann Arbor, Mich.). Nitrogen was the carrier gas (30 ml/min). Air (300 ml/min) and hydrogen (30 ml/min) were utilized in the flame ionization detector. The column was at 100°C, the detector at 320°C, and the injector port at 250°C. The wavelength for infrared (ir) analysis was 13.3 μm, slit width 1 mm, and path length 0.75 m.

Study design. Rats and mice were exposed to 200, 500, or 1000 ppm DCM for 8 hr/day for 5 days or 12 hr/day for 4 days (Fig. 1). The COHb levels were measured immediately following the first day's exposure, and prior to the second day's exposure to compare the effects of a single 8- or 12-hr exposure on COHb formation. Immediately after termination of the last exposure and on the following Monday morning, COHb levels in the two groups were compared to examine the weekly effect of the unusual exposure schedule. In experiments to determine the kinetics of COHb and DCM elimination, a single exposure was employed.

Measurements of COHb and DCM in blood. The COHb concentrations in blood were determined using a modification of the method of Rodkey *et al.* (1979). A blood sample obtained from the orbital sinus was diluted 1500-fold with 0.01 M Tris solution containing sodium dithionite to prevent dissociation of COHb by oxygen. Absorbance measurements were made at 420 and 432 nm in a Beckman Acta C III spectrophotometer (Beckman Instruments Inc., Irvine, Calif.). The fraction of the total hemoglobin present as COHb was calculated from these measurements using molar absorptivities of hemoglobin and carboxyhemoglobin determined in this laboratory.

The DCM levels in blood obtained from the orbital sinus were determined by gas chromatography. A blood sample was incubated in a vial at 65°C for 10 min before the head space vapor was injected into a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. The GC conditions were the same as those for the DCM chamber concentration measurements described above.

Data analyses. All results were analyzed by a two-tailed Student's t test, or a one- or two-way analysis of variance (ANOVA). The acceptable level of significance was established at $p < 0.05$. In the elimination studies, a half-life was derived from a semilogarithmic plot of concentration versus time by the method of least squares.

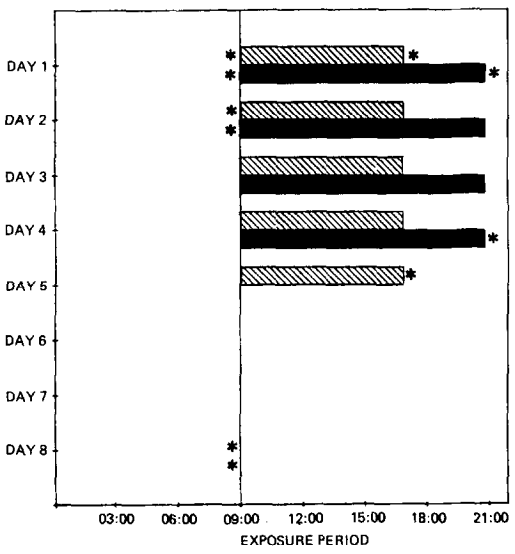

FIG. 1. Exposure schedules for rats and mice exposed to DCM. The cross-hatched bars represent exposure periods mimicking the standard workshift, black bars the unusual workshift. Asterisks indicate the times of COHb measurement.

RESULTS

COHb Formation from Repeated Exposure to DCM

The COHb level following a single 12-hr exposure was not significantly different ($p > 0.05$) from that of the 8-hr exposure regardless of the DCM concentration used either at the end of the exposure period or on the next morning (Fig. 2). Repeated exposures for 4 or 5 days did not result in an increase in the maximum COHb levels from the 1-day exposure value, indicating that there was no cumulative effect of COHb formation. No difference between the COHb levels in the two exposure groups was found after 1 week. Increasing the DCM concentration from 200 to 500 to 1000 ppm caused only slight changes in COHb levels. This is in agreement with the work of others, suggesting lack of a linear dose–response

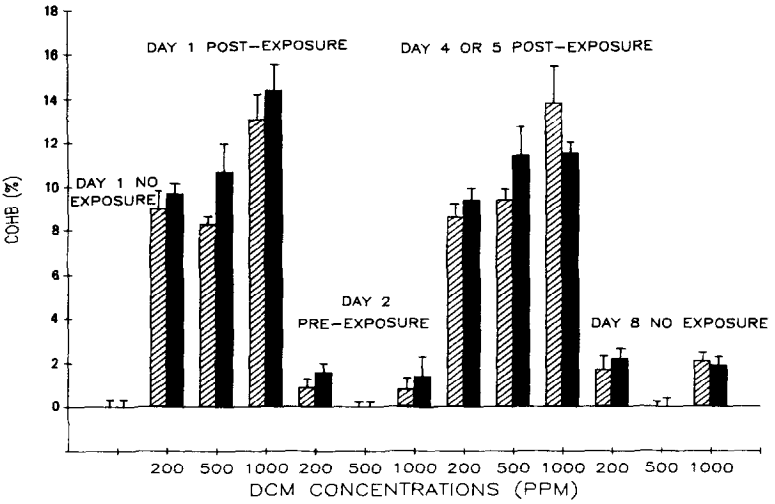

FIG. 2. Comparison of the effects of the standard and unusual exposure schedules on the COHb levels in rats exposed to DCM. The cross-hatched bar represents the standard exposure group, the black bar the unusual exposure group. Each value is mean ± SE for five rats. No significant difference ($p > 0.05$) in the COHb level between the two groups was observed at any time point.

relationship (Hogan *et al.*, 1976; Kurppa and Vainio, 1981; McKenna *et al.*, 1982).

In experiments using mice, similar results were obtained (Fig. 3). The COHb levels of the two exposure groups were not significantly different at the times of measurement. Repeated exposures failed to increase the maximum COHb levels in both groups. Increasing

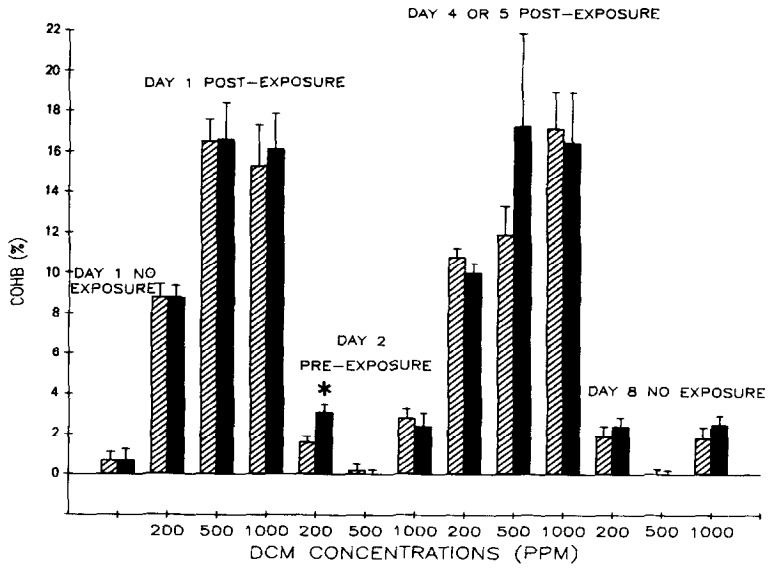

FIG. 3. Comparison of the effects of the standard and unusual exposure schedules on the COHb levels in mice exposed to DCM. The cross-hatched bar represents the standard exposure group, the black bar the unusual exposure group. Each value is mean ± SE for five mice. An asterisk indicates a significant difference ($p < 0.05$) in the COHb level between the two groups.

the DCM concentration from 500 to 1000 ppm did not alter the postexposure COHb level (two-way ANOVA, $p > 0.05$), suggesting that the metabolism of DCM to CO was saturated.

Decline of DCM and COHb in Rat Blood

Rats were exposed to 1000 ppm DCM for 8 or 12 hr, or to 500 ppm for 8 hr for 1 day. Dichloromethane was eliminated from blood with a half-life of approximately 20 min in all three groups (Fig. 4). Prolonging the exposure period from 8 to 12 hr did not affect the maximum blood DCM concentration or the rate of its elimination. Decreasing the DCM exposure concentration lowered the maximum DCM level in blood. However, the rate of DCM elimination was not affected.

The rate of disappearance of COHb following exposure to 550 ppm DCM for 8 or 12 hr is shown in Fig. 5. The COHb levels declined in a log-linear fashion with a half-life of approximately 50 min for both groups. There was no significant difference between the two groups in the rate of COHb decline or in the COHb level at any time point. Increasing the DCM exposure level to 960 ppm prolonged the half-life of COHb to approximately 130 min. However, no significant difference between the 8- and 12-hr exposure groups was noted. The maximum COHb level was not altered by increasing the exposure concentration, again suggesting saturation of the biotransformation of DCM to CO.

Effect of a Drug Metabolism Inhibitor on CO and COHb Disappearance from Rat Blood

Attempts were made to alter the metabolism of DCM in order to examine the influence of decreasing the conversion of DCM to CO on the rates of DCM and COHb disappearance from blood. Preliminary experiments with SKF 525-A and cobaltous chloride indicated that these agents had no effect. However, pyrazole effectively decreased the steady-state COHb level and increased the DCM level in blood (Fig. 6).

The effect of increasing the exposure period from 8 to 12 hr on the rates of COHb and

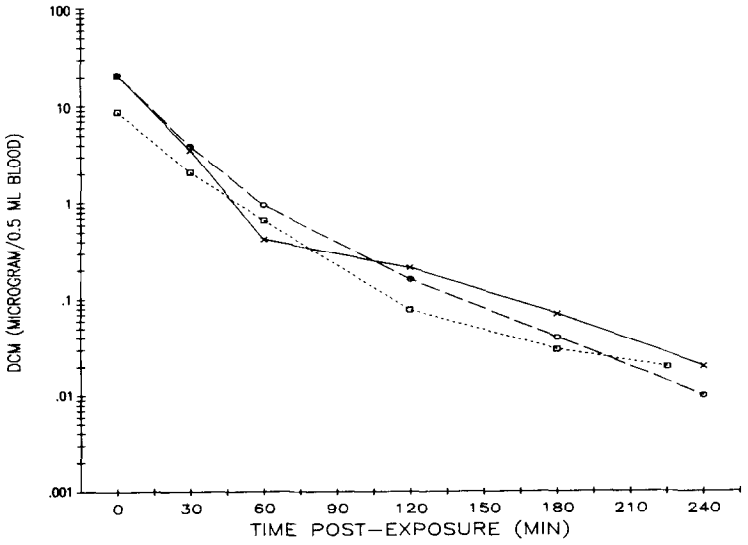

FIG. 4. Elimination of DCM from blood in rats. Following the exposure to 1070 ppm DCM for 8 or 12 hr, the blood DCM level was measured at the indicated time points. An × is the mean for five rats exposed for 8 hr. A circle is the mean for five rats exposed for 12 hr. A square is the mean for four to nine rats exposed to 500 ppm DCM for 8 hr. Standard errors were omitted for the sake of clarity.

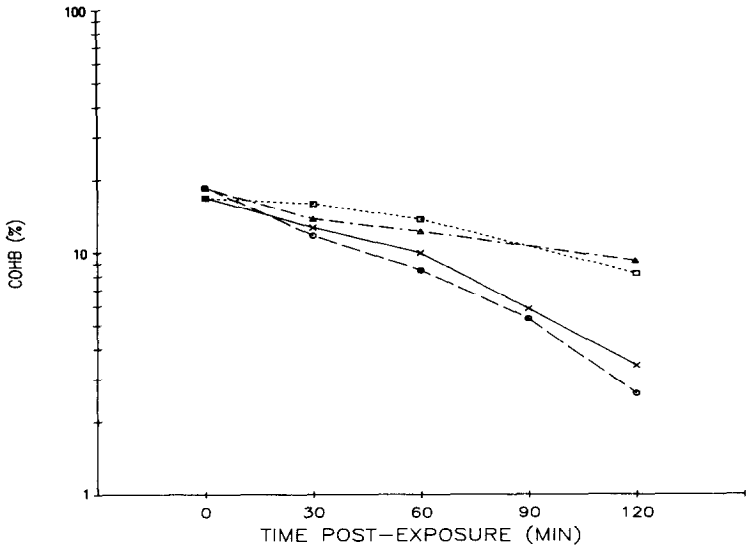

FIG. 5. Disappearance of COHb from blood in rats. Rats were exposed to 550 or 960 ppm DCM for either 8 or 12 hr. Following the exposure, the COHb level was measured. An × is the mean for five rats exposed to 550 ppm for 8 hr. A circle is the mean for five rats exposed for 12 hr. A square is the mean for five rats exposed to 960 ppm DCM for 8 hr. A triangle is the mean for five rats exposed for 12 hr. There was no significant difference ($p > 0.05$) in the COHb level between the two groups exposed to the same DCM exposure concentration for different periods. Standard errors were omitted for the sake of clarity.

DCM disappearance in pyrazole-treated rats is shown in Fig. 7. The presence of COHb and DCM in blood was extended in pyrazole-treated rats compared to the previous observations in control rats, but the rates of disappearance were not affected by increasing the exposure period from 8 to 12 hr (Student's t test, $p > 0.05$).

Effect of the Unusual Exposure Schedule in Pyrazole-Treated Rats

Rats were treated with pyrazole, prior to the initiation of each exposure, and exposed to 500 ppm DCM for 8 hr/day for 5 days or 12 hr/day for 4 days. The COHb levels were measured twice a day, immediately prior to and following the exposure. The COHb levels in rats on the unusual exposure schedule were not significantly different from those in rats on the standard schedule (Fig. 8). Some cumulative effect was noted from the first to the second day of exposure in both groups.

DISCUSSION

The present studies indicate that DCM gains access to the body very rapidly, probably because of its relatively high solubility in blood, and is rapidly eliminated. This is supported by the failure of an increase in the DCM exposure level or prolongation of the exposure period to alter the rate of DCM elimination from blood. The short half-life of DCM in blood suggests that cumulative effects of DCM exposure would be minimal.

No cumulative effect of COHb was noted in rats repeatedly exposed to DCM for 8 or 12 hr/day. In the single exposure studies, the COHb levels for both the 8- and 12-hr groups at both 550 and 960 ppm DCM were similar, probably due to saturation of the enzymatic pathway. Increasing the DCM exposure level resulted in the persistent presence of COHb in blood. It is speculated that the COHb persists longer in the high DCM exposure level because the DCM available for the metabolic conversion to CO would last longer. This is in

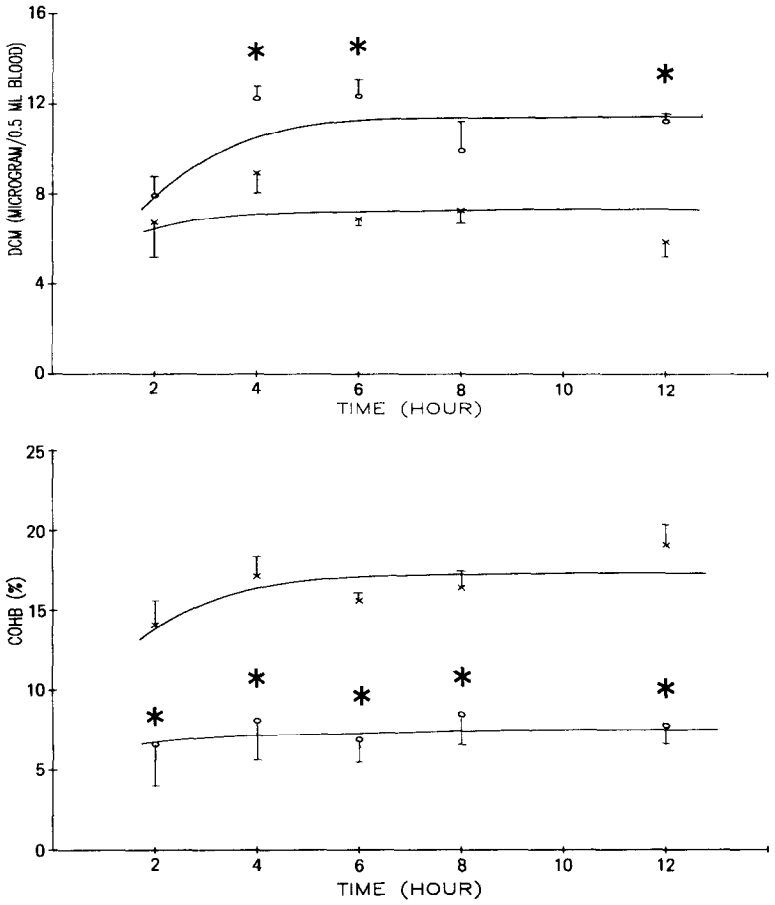

FIG. 6. Effect of pyrazole on DCM and COHb levels in rats under DCM exposure. Rats were treated with pyrazole (300 mg/kg, ip) 15 min prior to the exposure to 510 ppm DCM. The DCM and COHb concentrations in blood were measured during exposure. An × is the mean ± SE for four control rats. A circle is the mean ± SE for four rats treated with pyrazole. An asterisk indicates a significant difference ($p < 0.05$) from the control.

general agreement with the pharmacokinetic behavior of metabolites of inhaled chemicals (Andersen, 1981). The failure to observe a dose–response relationship between exposure concentrations and COHb levels in the present investigation is in good agreement with earlier work of others.

The effectiveness of pyrazole on the metabolism of DCM should be attributed either to higher potency of pyrazole on the enzyme(s) responsible for the CO formation or to the possible selectivity of drug metabolism inhibitors on multiple isozymes of cytochrome *P*-450 in the mixed-function oxidase system.

Repeated exposures to DCM for 8 or 12 hr daily of rats treated with pyrazole every day resulted in an increase in the maximum COHb on the second day and the resultant residual COHb on the next morning. However, this cumulative effect was absent from the third day on. Since pyrazole is strongly hepatotoxic (Magnusson *et al.*, 1972; MacDonald *et al.*, 1981), the liver damage due to repeated pyrazole treatment might lead to a decrease in the activity of enzyme systems responsible for the metabolic conversion of DCM to CO.

The objective of the present studies was to use an animal model to examine the possible

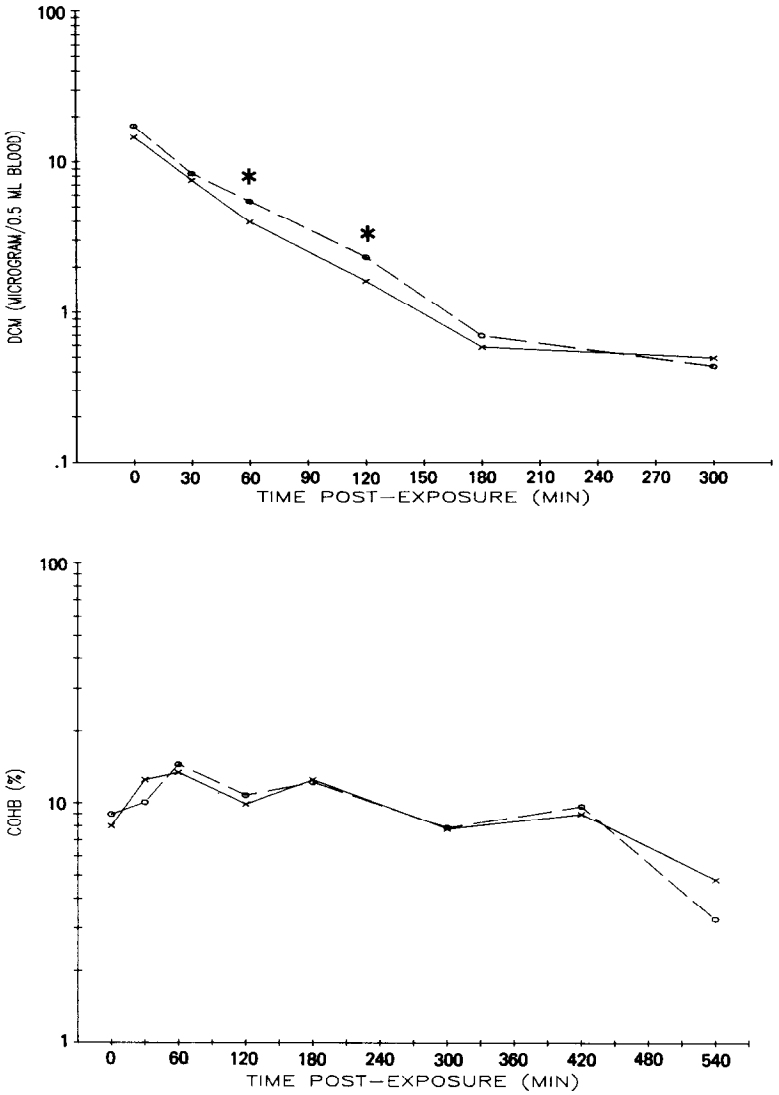

FIG. 7. Effect of pyrazole on the rate of DCM and COHb disappearance from rat blood. Rats were treated with pyrazole (300 mg/kg, ip) $12\frac{1}{4}$ hr prior to the cessation of the exposure to 530 ppm DCM for 8 or 12 hr. Following the exposure, the DCM and COHb concentrations in blood were measured. An × is the mean for five rats exposed for 8 hr. A circle is the mean for five rats exposed for 12 hr. An asterisk indicates a significant difference ($p < 0.05$) between the blood levels of DCM in the two groups. However, there was no significant difference ($p > 0.05$) between the two groups in the rate of DCM or COHb elimination. Standard errors were omitted for the sake of clarity.

increase in the susceptibility of individuals to an industrial chemical when working a 12-hr workshift. The effect of the 12-hr exposure schedule on the COHb level was not significantly different from that of the 8-hr exposure schedule when rats and mice were exposed to the same concentration of DCM. In both the 8- and 12-hr exposure groups there was a similar rapid elimination of DCM from blood. It is generally accepted that the intensity of toxic response is a function of the concentration of the toxic agent that reaches the site of action

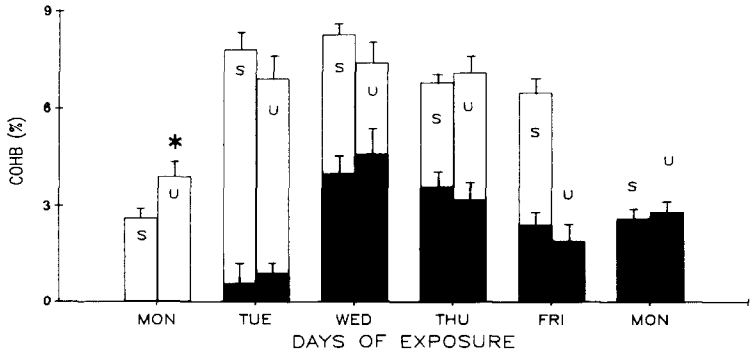

FIG. 8. Effect of the unusual exposure schedule on the COHb level induced by DCM exposure in pyrazole-treated rats. Rats were treated with pyrazole (150 mg/kg, ip) daily for 5 days, and immediately following the treatment, exposed to 500 ppm DCM for either 8 hr/day for 5 days (S) or 12 hr/day for 4 days (U). The COHb levels were measured twice in a day, immediately before (black bar) and after (the total height of black and white bars) the exposure. Each value given is mean ± SE for six rats. An asterisk indicates a significant difference ($p < 0.05$) between the two groups.

(Amdur, 1973). Therefore, it may be concluded that for a compound such as DCM, with a short biological half-life and readily reversible toxic effect, no adjustment of the current exposure limit would be necessary for a longer workshift. This supports the acceptance of the mathematical models (Mason and Dershin, 1976; Hickey and Reist, 1977; Roach, 1978) based upon the pharmacokinetics of a toxicant. The earlier models (Brief and Scala, 1975; OSHA, 1979), which were based solely on the work hours and the recovery period, suggest reduction of the current exposure limits to the same extent for a given unusual workshift regardless of the nature of each substance. However, final extrapolation of these results with rats and mice to occupational human exposure to DCM needs to be reserved because of differences in the half-life of COHb between human and rodents. Ratney *et al.* (1974) estimated that the half-life of COHb in humans exposed to DCM was 13 hr. The half-life of 13 hr indicates that there would be significant cumulative effect induced by DCM exposure if a workday were lengthened from 8 to 12 hr and the recovery shortened from 16 to 12 hr. Another factor to be considered is that workers may face an additional burden of CO because of its ubiquitous nature in the environment. In a study on the additivity of exogenous CO and that formed from DCM, Kurppa *et al.* (1981) observed that COHb levels resulting from exposure to 1000 ppm DCM and 100 ppm CO were additive in rats.

ACKNOWLEDGMENTS

This work was supported in part by BRSG 2SO7RR05586 awarded by Division of Research Resources, National Institutes of Health, and by OH01531 awarded by National Institute for Occupational Safety and Health.

REFERENCES

AHMED, A. E., AND ANDERS, M. W. (1976). Metabolism of dihalomethanes to formaldehyde and inorganic halide. *Drug Metab. Dispos.* **4,** 357–361.

AHMED, A. E., AND ANDERS, M. W. (1978). Metabolism of dihalomethanes to formaldehyde and inorganic halide. II. Studies on the mechanism of the reaction. *Biochem. Pharmacol.* **27,** 2021–2025.

AMDUR, M. O. (1973). Industrial toxicology. In *The Industrial Environment—Its Evaluation and Control.* National Institute for Occupational Safety and Health, Rockville, Md.

American Conference of Governmental Industrial Hygienists (ACGIH) (1984). *Threshold Limit Values for Chemical Substances in the Work Environment Adopted by ACGIH for 1984–1985. ACGIH, Cincinnati, Ohio.*

ANDERSEN, M. E. (1981). Saturable metabolism and its relationship to toxicity. *CRC Crit. Rev. Toxicol.* **9,** 105–150.

BRIEF, R. S., AND SCALA, R. A. (1975). Occupational ex-

posure limits for novel work schedules. *Amer. Ind. Hyg. Assoc. J.* **36,** 467–471.

HEPPEL, L. A., NEAL, P. A., PERRIN, T. L., ORR, M. L., AND POTERFIELD, V. T. (1944). Toxicology of dichloromethane (methylene chloride). I. Studies on effects of daily inhalation. *J. Ind. Hyg. Toxicol.* **26,** 8–16.

HICKEY, J. L. S., AND REIST, C. (1977). Application of occupational exposure limits to unusual work schedules. *Amer. Ind. Hyg. Assoc. J.* **38,** 613–621.

HOGAN, G. K., SMITH, R. G., AND MCLAUGHLIN, J. M. (1976). Studies on the microsomal conversion of dichloromethane to carbon monoxide. *Toxicol. Appl. Pharmacol.* **37,** 112.

KLAASSEN, C. D., AND PLAA, G. L. (1966). Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. *Toxicol. Appl. Pharmacol.* **9,** 139–151.

KUBIC, V. L., AND ANDERS, M. W. (1975). Metabolism of dihalomethanes to carbon monoxide. II. In vitro studies. *Drug Metab. Dispos.* **3,** 104–111.

KUBIC, V. L., AND ANDERS, M. W. (1978). Metabolism of dihalomethanes to carbon monoxide. III. Studies on the mechanism of the reaction. *Biochem. Pharmacol.* **27,** 2349–2355.

KUBIC, V. L., ANDERS, M. W., ENGEL, R. R., BARLOW, C. H., AND CAUGHEY, W. S. (1974). Metabolism of dihalomethanes to carbon monoxide. I. In vivo studies. *Drug Metab. Dispos.* **2,** 53–57.

KURPPA, K., KIVISTO, H., AND VAINIO, H. (1981). Dichloromethane and carbon monoxide inhalation: Carboxyhemoglobin addition, and drug metabolizing enzymes in rat. *Int. Arch. Occup. Environ. Health* **49,** 83–87.

KURPPA, K., AND VAINIO, H. (1981). Effects of intermittent dichloromethane inhalation on blood carboxyhemoglobin concentration and drug metabolizing enzymes in rat. *Res. Commun. Chem. Pathol. Pharmacol.* **32,** 535–544.

LANGEHENNIG, P. L., SEELER, R. A., AND BERMAN, E. (1976). Paint removers and carboxyhemoglobin. *N. Engl. J. Med.* **295,** 1137.

MACDONALD, E., IHALAINEN, E., AND PISPA, J. P. (1981). Pharmacological and toxicological properties of 4-hydroxypyrazole, a metabolite of pyrazole. *Acta Pharmacol. Toxicol.* **48,** 418–423.

MAGNUSSON, G., NYBERG, J. A., BODIN, N. O., AND HANSSON, E. (1972). Toxicity of pyrazole and 4-methylpyrazole in mice and rats. *Experientia* **28,** 1198–1200.

MASON, J. W., AND DERSHIN, H. (1976). Limits to occupational exposure in chemical environments under novel work schedules. *J. Occup. Med.* **18,** 603–607.

MCKENNA, M. J., SAUNDERS, J. H., BOECKLER, W. H., KARBOWSKI, R. J., NITSCHKE, K. D., AND CHENOWETH, M. B. (1980). *The Pharmacokinetics of Inhaled Methylene Chloride in Human Volunteers,* Abstract No. 176, Presented at the 19th annual meeting of the Society of Toxicology, Washington, D.C.

MCKENNA, M. J., AND ZEMPEL, J. A. (1981). The dose-dependent metabolism of [^{14}C]methylene chloride following oral administration to rats. *Food Cosmet. Toxicol.* **19,** 73–78.

MCKENNA, M. J., ZEMPEL, J. A., AND BRAUN, W. H. (1982). The pharmacokinetics of inhaled methylene chloride in rats. *Toxicol. Appl. Pharmacol.* **65,** 1–10.

Occupational Safety and Health Administration (OSHA) (1979). *Compliance Officers: Field Manual.* U.S. Department of Labor, Washington, D.C.

PAUSTENBACH, D. J. (1982). *The Effect of the Twelve-Hour Workshift on the Toxicology, Disposition and Pharmacokinetics of Carbon Tetrachloride in the Rat.* Doctoral dissertation, Purdue University, West Lafayette, Ind.

PETERSON, J. E., AND STEWART, R. D. (1970). Absorption and elimination of carbon monoxide by inactive young men. *Arch. Environ. Health* **21,** 165–171.

PLAA, G. L. (1973). Quantitative comparison of the hepatotoxic potencies of various halogenated hydrocarbons. In *Adverse Effects of Environmental Chemicals and Psychotropic Drugs* (M. Horvath, ed.), Vol. 1., Elsevier, Amsterdam.

RATNEY, R. S., WEGMAN, D. H., AND ELKINS, H. B. (1974). In vivo conversion of methylene chloride to carbon monoxide. *Arch. Environ. Health* **28,** 223–226.

ROACH, S. A. (1978). Threshold limit values for extraordinary work schedules. *Amer. Ind. Hyg. Assoc. J.* **39,** 345–364.

RODKEY, F. L., HILL, T. A., PITTS, L. L., AND ROBERTSON, R. F. (1979). Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood. *Clin. Chem.* **25,** 1388–1393.

STEWART, R. D., FISHER, T. N., HOSKO, M. J., PETERSON, J. E., BARETTA, E. D., AND DODD, H. C. (1972a). Carboxyhemoglobin elevation after exposure to methylene chloride. *Science (Washington, D.C.)* **176,** 295–296.

STEWART, R. D., FISHER, T. N., HOSKO, M. J., PETERSON, J. E., BARRETA, E. D., AND DODD, H. C. (1972b). Experimental human exposure to methylene chloride. *Arch. Environ. Health* **25,** 342–348.

VENG-PEDERSEN, P. (1984). Pulmonary absorption and excretion of compounds in the gas phase. Theoretical pharmacokinetic and toxicokinetic analysis. *J. Pharm. Sci.* **73,** 1136–1141.

VON OETTINGEN, W. F. (1964). *The Halogenated Hydrocarbons of Industrial and Toxicological Importance.* Elsevier, Amsterdam.

WILSON, J. T., AND ROSE, K. M. (1978). *The Twelve-Hour Shift in the Petroleum and Chemical Industries of the United States and Canada: A Study of Current Experience.* Wharton Business School, University of Pennsylvania, Philadelphia.