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Influence of exposure mode on vinyl chloride action

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Abstract. Rats were subjected to 4 h continuous or intermittent exposure to vinyl chloride (VC) at different time-weighted average concentrations (15, 50, 150, 500 and 15,000 mg/m³). Hepatic non-protein sulfhydryl content (NPSH) and excretion of thiodiglycolic acid (TdGA) in urine were determined. VC at concentrations from 50 mg/m³ to 15,000 mg/m³ caused a dose-dependent depression of NPSH, but no difference in the magnitude of this depression induced by continuous or intermittent exposure at the same average concentration of VC was noted. At average concentrations of 50 mg/m³ and 150 mg/m³, the urinary excretion of TdGA under continuous exposure did not differ from that under intermittent exposure, whereas at VC concentrations of 500 mg/m³ and 15,000 mg/m³ it was higher following continuous exposure.

Key words: Vinyl chloride – Sulfhydryl groups – Thiodiglycolid acid – Continuous and intermittent exposure

Introduction

During vinyl chloride (VC) or polyvinyl chloride (PVC) production, the concentrations of VC in the air are subject to notable changes and thus the workers may be exposed not to constant but to variable concentrations of this monomer. Some groups of workers, e.g. cleaners, may periodically be exposed to very high concentration of VC, several times greater than the MAC value.

The influence of the exposure mode on VC toxicity has not been investigated. In experimental studies of this subject, it would be advisable to take into consideration the main direction of the adverse effects of VC under actual levels of professional exposure, carcinogenicity and toxic injury of the liver, which arise as a consequence of chronic inhalation. However, such studies performed on animals are long-term and very expensive. Thus, we first undertook model studies of acute exposure, seeking in the results of these investigations grounds for initiating chronic studies.

From animal studies it is obvious that the rate of VC metabolism is dependent on the level of exposure (Hefner et al. 1975; Gehring et al. 1978; Watanabe et al.1978b; Filser and Bolt 1979). Hence, the type of VC exposure may differentiate the toxicity induced by this monomer. Bolt et al. (1981), on the basis of kinetic considerations, suggested that toxic effects with continuous and intermittent exposure should not differ at the same average concentration of VC, but experimental evidence is lacking.

The most commonly postulated reactive VC metabolite has been chloroethylene oxide (Barbin et al. 1975; Bartsch et al.1979; Guengerich et al. 1979, 1981; Zajdela et al. 1980). This epoxide rearranges to chloroacetaldehyde, which can also react with tissue nucleophiles (Guengerich et al. 1979, 1981) and lead to mutations (Malaveille et al. 1975; McCann et al.1975). It is assumed that the primary detoxication mechanism of VC involves conjugation with hepatic glutathione followed by the modification of the peptide moiety, leading to sulfur-containing urinary excretion products (Green and Hathway 1975, 1977; Watanabe et al.1976a, b, c, 1978a, b; Müller and Norpoth 1975; Müller et al. 1976, 1978, 1979).

Our studies aim to compare the effect of acute continuous and intermittent exposure of rats to VC on hepatic non-protein sulfhydryl content (NPSH) and urinary excretion of thiodiglycolic acid (TdGA), one of the major VC metabolites.

Materials and methods

Male Wistar rats, body weight 250 ± 25 g, were subjected to 4 h continuous or intermittent exposure to VC at the same time-weighted average concentrations of 15, 50, 150, 500 and 15,000 mg/m³ in toxicological chambers of 0.25 m³ volume. Unter continuous exposure, the VC concentrations were constant during 4 h inhalation. The intermittent exposure involved five 15 min peak concentrations of VC and four 40 min intervals without inhalation of VC (Fig. 1). Control

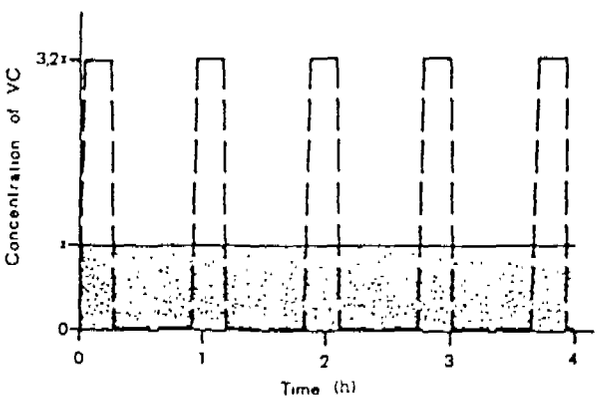

Fig. 1. Time course of VC concentration under continuous (*solid line*) or intermittent (*dashed line*) exposure

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animals were placed in the chamber for 4 h without exposure to VC. VC was determined by gas chromatography according to the method of Krajewski and Dobecki (1978). The time-weighted average analytical concentrations of VC under continuous exposure were: 14.7 ± 1.4 (±SD), 49.6 ± 2.1, 149 ± 11, 560 ± 28, and 15,275 ± 228 mg/m^3, and under intermittent exposure: 14.4 ± 1.4, 46.5 ± 4.8, 149 ± 13, 507 ± 23, and 15,194 ± 466 mg/m^3 (peak concentrations of VC were: 46.1, 148.8, 477, 1,622, and 48,621 mg/m^3, respectively).

NPSH was determined in the 9,000 *g* supernatant of liver immediately after termination of exposure, and then after 20 h and 44 h from the beginning, according to the method of Ellman (1959).

The urinary excretion of TdGA was determined in VC-exposed rats by the method of Dramiński and Trojanowska (1981) with some modifications: (1) instead of three-fold extraction with 10 ml ethyl acetate, the urine was extracted twice with 20 ml ethyl acetate; (2) a Varian 1400 gas chromatograph equipped with flame ionization detector and glass column 1.8 m long (internal diameter 2 mm) packed with 3% OV-1 on Gas-Chrom Q, 100/120 mesh was used. Retention times of TdGA and *o*-phthalic acid, the latter used as an internal standard, were about 6 and 10 min, respectively; (3) injector temperature was 235° C and detector temperature 285° C; (4) a 3 µl urine specimen silylated with 0.2 ml trimethylsilyldiethylamine-pirydyne (1 : 1) mixture (after standing at room temperature for 30 min) was injected onto the column.

Results were statistically evaluated according to the Student's *t*-test.

Results

The content of NPSH in the liver of rats subjected to acute continuous or intermittent exposure to VC is presented in Table 1. No statistically significant differences were found between continuous and intermittent exposure at the same time-weighted average concentrations of VC. Following both types of exposure, similar dose-dependent depression of hepatic NPSH was observed at concentrations of 50–15,000 mg/m^3 immediately after termination of exposure. After 20 h the NPSH level was still 13–14% lower at the highest concentration of VC (15,000 mg/m^3) than in the controls, whereas a slight (9–12%) but statistically significant increase in hepatic NPSH was observed in rats exposed to VC at a concentration of 500 mg/m^3. After 44 h, the hepatic NPSH in VC-exposed animals did not differ from the control.

The excretion of TdGA in the urine of rats subjected to acute continuous or intermittent exposure to VC is shown in

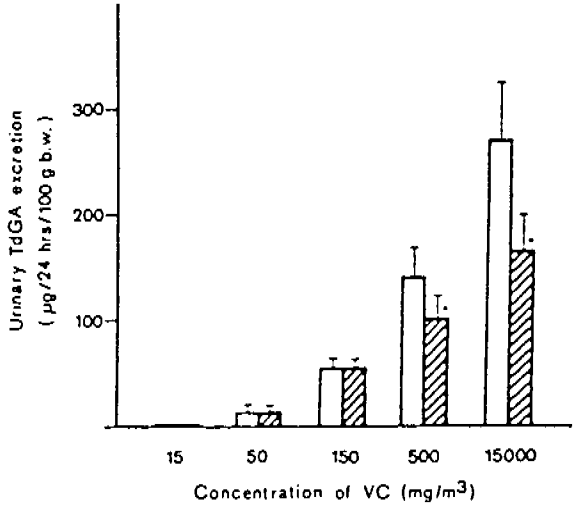

Fig. 2. Urinary TdGA excretion following 4 h continuous or intermittent exposure to VC. *Open bars* represent continuous exposure and *hatched bars* intermittent. Data are mean values ± SD from six determinations. * Difference statistically significant in relation to continuous exposed group, $P < 0.05$

Table 1. Hepatic non-protein sulfhydryl content following 4 h continuous or intermittent exposures to VC

Experimental group	Concentration of VC (mg/m^3)	Hepatic non-protein sulfhydryl content (µM SH/g liver)		
		Immediately after exposure	20 h after exposure	44 h after exposure
Control	0	5.54 ± 0.41	5.82 ± 0.40	5.62 ± 0.28
Continuous	15	5.52 ± 0.36	5.76 ± 0.30	5.55 ± 0.24
Intermittent	15[a]	5.57 ± 0.36	5.73 ± 0.39	5.61 ± 0.28
Control	0	5.68 ± 0.20	5.87 ± 0.31	5.69 ± 0.21
Continuous	50	5.21 ± 0.25[b]	5.98 ± 0.36	5.79 ± 0.19
Intermittent	50[a]	5.31 ± 0.25[b]	5.87 ± 0.31	5.76 ± 0.19
Control	0	5.46 ± 0.37	5.51 ± 0.30	5.35 ± 0.71
Continuous	150	4.31 ± 0.28[b]	5.18 ± 0.46	5.31 ± 0.41
Intermittent	150[a]	4.28 ± 0.34[b]	5.29 ± 0.28	5.32 ± 0.36
Control	0	5.56 ± 0.53	5.44 ± 0.33	5.53 ± 0.28
Continuous	500	3.98 ± 0.45[b]	5.93 ± 0.35[b]	5.22 ± 0.24[b]
Intermittent	500[a]	4.02 ± 0.46[b]	6.09 ± 0.41[b]	5.57 ± 0.42
Control	0	6.02 ± 0.25	5.83 ± 0.18	5.82 ± 0.30
Continuous	15,000	2.50 ± 0.18[b]	5.00 ± 0.45[b]	5.91 ± 0.68
Intermittent	15,000[a]	2.36 ± 0.24[b]	5.07 ± 0.64[b]	5.93 ± 0.37

Values are means ± SD from eight determinations

[a] Time-weighted average concentration of VC

[b] Difference statistically significant in relation to control, $P < 0.05$

Fig. 2. Different results were obtained depending on the concentration of VC. At concentrations of 50 mg/m³ and 150 mg/m³ no differences in urinary excretion of TdGA were found between continuous and intermittent exposure. On the other hand, at concentrations of 500 mg/m³ and 15,000 mg/m³ the excretion of TdGA in the urine of rats after continuous exposure was respectively 39% and 62.5% higher than after intermittent exposure.

Discussion

Several authors have proved that exposure to VC was followed by hepatic NPSH depression (Hefner et al.1975; Watanabe et al. 1976a, 1978a; Tarkowski et al. 1980; Wiśniewska-Knypl et al.1981). Our results showed that exposure to VC in the concentration range from 50 mg/m³ to 15,000 mg/m³ led to a dose-dependent decrease in NPSH level in the liver of rats, and the magnitude of this depression did not differ between continuous and intermittent exposure at the same time-weighted concentration of VC. Under both continuous and intermittent exposure to VC at an average concentration of 15 mg/m³, depression of NPSH was not observed. This suggests that the VC threshold concentration for depletion of NPSH in rats liver lies in the range between 15 mg/m³ and 50 mg/m³.

TdGA, one of the major final products of VC metabolism (Green and Hathway 1975, 1977; Watanabe et al. 1976b, c, 1978a; Müller and Norpoth 1975; Müller et al. 1976, 1978, 1979) could be determined by the method used in our studies in 24 h urine after exposure of rats to VC at concentrations from 50 mg/m³ to 15,000 mg/m³. In the logarithmic plot, the urinary excretion of TdGA increased almost linearly with concentration of VC up to 500 mg/m³ in a single 4 h continuous exposure (Fig. 3). Evident deviation from linearity was observed with a high concentration of VC (15,000 mg/m³) both in continuous and intermittent exposures. This phenomenon can be explained by saturation of the VC metabolizing enzymes at higher concentrations of VC, as shown by Gehring et al. (1978) and Filser and Bolt (1979).

At concentrations of 500 mg/m³ and 15,000 mg/m³ the urinary excretion of TdGA was lower after intermittent exposure, as compared with continuous exposure, in spite of comparable depletion of hepatic NPSH. The mechanism underlying these findings is not clear. Three possible explanations may be considered: (1) lower rate of total VC metabolism unter intermittent exposure as compared to continuous; (2) changing proportions of common urinary metabolites of VC as a result of different concentrations of VC under continuous or intermittent exposure; and (3) differe tissue binding of VC reactive metabolites under continuou exposure as compared to that under intermittent exposure.

In practical situations of industrial and environmental exposures the results obtained with lower concentrations of VC should be primarily taken into consideration. Taking into account the rate of VC metabolism at concentrations not exceeding 150 mg/m³, the toxic effects of VC following continuous or intermittent exposure should not differ, assuming that the average time-weighted concentration of VC with both types of exposure is comparable.

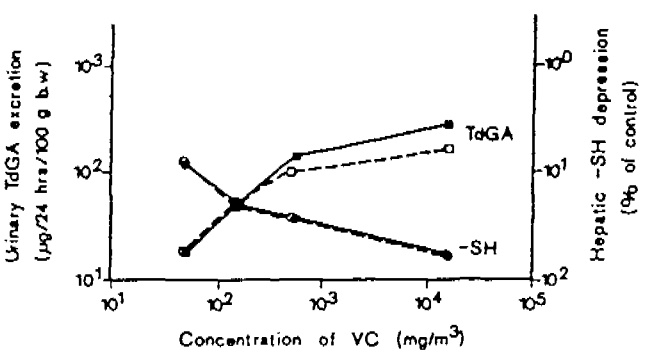

Fig. 3. Urinary TdGA excretion and depression of hepatic non-protein sulfhydryl content in rats following continuous or intermittent exposures to VC in relation to the concentration of inhaled VC. Solid lines represent continuous exposure and dashed lines intermittent. □ ■ TdGA; ○ NPSH

References

Barbin A, Bresil H, Croisy A, Jacquignon P, Montesano R, Bartsch H (1975) Liver microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. Biochem Biophys Res Commun 67: 596–603

Bartsch H, Malaveille C, Barbin A, Planche G (1979) Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes, and dichlorobutadienes produced by rodent or human liver tissue: evidence for oxirane formation by P450-linked microsomal mono-oxygenases. Arch Toxicol 41: 249–277

Bolt HM, Filser JG, Buchter A (1981) Inhalation pharmacokinetics based on gas uptake studies. III A pharmacokinetic assessment in man of "peak concentrations" of vinyl chloride. Arch Toxicol 48: 213–228

Dramiński W, Trojanowska B (1981) Chromatographic determination of thiodiglycolic acid – a metabolite of vinyl chloride. Arch Toxicol 48: 289–292

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70–77

Filser JG, Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42: 123–136

Gehring PJ, Watanabe PG, Park CN (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation: Example – vinyl chloride. Toxicol Appl Pharmacol 44: 581–591

Green T, Hathway DE (1975) The biological fate in rats of vinyl chloride in relation to its oncogenicity. Chem Biol Interact 11: 545–562

Green T, Hathway DE (1977) The chemistry and biogenesis of the S-containing metabolites of vinyl chloride in rats. Chem Biol Interact 17: 137–150

Guengerich FP, Crawford WM, Watanabe PG (1979) Activation of vinyl chloride to covalently bound metabolites: roles of 2-chloroethylene oxide and 2-chloroacetaldehyde. Biochemistry 18: 5177–5182

Guengerich FP, Mason PS, Stott WT, Fox TR, Watanabe PG (1981) Roles of 2-haloethylene oxides and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in inreversible binding to protein and DNA. Cancer Res 41: 4391–4398

Hefner RE, Watanabe PG, Gehring PJ (1975) Preliminary studies of the fate of inhaled vinyl chloride monomer in rats. Ann N Y Acad Sci 246: 135–148

Krajewski J, Dobecki M (1978) Determination of vinyl chloride in the air by gas chromatographic method (in Polish). Med Pracy 29: 403–410

Malaveille C, Bartsch H, Barbin A, Camus AM, Montesano R (1975) Mutagenicity of vinyl chloride, chloroethyleneoxide, chloroacetaldehyde and chloroethanol. Biochem Biophys Res Comm 63: 363–370

McCann J, Simmon V, Streitwieser D, Ames BH (1975) Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene

chlorohydrine), vinyl chloride, and cyclophosphamide. Proc Natl Acad Sci USA 72: 3190–3193

Müller G, Norpoth K (1975) Bestimmung zweier Urinmetabolite des Vinylchlorids. Naturwissenschaften 62: 541

Müller G, Norpoth K, Eckard R (1976) Identification of two urine metabolites of vinyl chloride by GC-MS-investigations. Int Arch Occup Environ Health 38: 69–75

Müller G, Norpoth K, Kusters E, Herweg K, Versin E (1978) Determination of thiodiglycolic acid in urine specimens of vinyl chloride exposed workers. Int Arch Occup Environ Health 41: 199–205

Müller G, Norpoth K, Wickramasinghe RH (1979) An analitical method, using GC-MS, for the quantitative determination of urinary thiodiglycolic acid. Int Arch Occup Environ Health 44: 185–191

Tarkowski S, Wiśniewska-Knypl JM, Klimczak J, Dramiński W, Wróblewska K (1980) Urinary excretion of thiodiglycolic acid and hepatic content of free thiols in rats at different levels of exposure to vinyl chloride. J Hyg Epidemiol Microbiol Immunol 24: 253–261

Watanabe PG, Hefner RE, Gehring PJ (1976a) Vinyl chloride induced depression of hepatic non-protein sulfhydryl content and effects on bromosulphalein (BPS) clearence in rats. Toxicology 6: 1–8

Watanabe PG, McGowan GR, Gehring PJ (1976b) Fate of (^{14}C) vinyl chloride after single oral administration in rats. Toxicol Appl Pharmacol 36: 339–352

Watanabe PG, McGowan GR, Madrid EO, Gehring PJ (1976c) Fate of (^{14}C) vinyl chloride following inhalation exposure in rats. Toxicol Appl Pharmacol 37: 49–59

Watanabe PG, Zempel JA, Gehring PJ (1978a) Comparison of the fate of vinyl chloride following single and repeated exposure in rats. Toxicol Appl Pharmacol 44: 391–399

Watanabe PG, Zempel JA, Pegg DG, Gehring PJ (1978b) Hepatic macromolecular binding following exposure to vinyl chloride. Toxicol Appl Pharmacol 44: 571–579

Wiśniewska-Knypl JM, Sokal JA, Klimczak J, Dajniak A, Bogdanikowa B (1981) Protective effect of methionine against vinyl chloride-mediated depression of non-protein sulfhydryls and cytochrome P-450. Toxicol Lett 8: 147–152

Zajdela F, Croisy A, Barbin A, Malaveille C, Tomatis L, Bartsch H (1980) Carcinogenicity of chloroethylene oxide, an ultimate reactive metabolite of vinyl chloride, and bis (chloromethyl) ether after subcutaneous administration and in initiation-promotion experiments in mice. Cancer Res 40: 352–356

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