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# Vinyl Chloride: An Example for Evaluating Mutagenic Effects in Mammals in vivo after Exposure to Inhalation

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**Abstract.** As part of a programme of investigations on the mutagenic effects in mammals in vivo after inhalation of environmental chemicals, the effects of the industrial compound vinyl chloride (VC) was analysed.

Chinese hamsters were exposed to 1.25%, 2.5% or 5% (v/v) VC in air for 6, 12 or 24 h. Bone marrow chromosomes were analysed for induced chromosome aberrations and sister-chromatid-exchanges (SCEs) 26 h after beginning of exposure.

The frequency of VC-induced chromosome aberrations and SCEs both depend on dose and length of exposure. The highest measured effects were 33.25 SCEs/cell after an exposure to 2.5% VC for 24 h and 25.7% metaphases with aberrations, when exposed to 5% VC for 24 h.

**Key words:** Vinyl chloride – Inhalation – Chinese hamsters – Chromosome aberrations – SCEs in vivo.

**Zusammenfassung.** Im Rahmen eines Programmes zur Untersuchung mutagener Effekte beim Säuger in vivo nach Inhalation von Umweltschadstoffen wurde Vinylchlorid (VC) untersucht.

Chinesische Hamster wurden mit 1,25%, 2,5% oder 5% (v/v) VC in Luft für 6, 12 oder 24 h begast. Chromosomen des Knochenmarks wurden 26 h nach Begasungsbeginn präpariert und auf induzierte Chromosomenaberrationen und Schwesterchromatid-Austausche (SCEs) hin untersucht.

Die Häufigkeit VC-induzierter Chromosomenaberrationen und SCEs hängt sowohl von der Dosis ab, als auch von der Expositionszeit. Die höchsten ermittelten Werte waren 33,25 SCEs/Zelle nach einer 24stündigen Begasung mit 2,5% VC und 25,7% Metaphasen mit Aberrationen nach 24stündiger Begasung mit 5% VC.

**Schlüsselwörter:** Vinylchlorid – Inhalation – Chinesische Hamster – Chromosomenaberrationen – SCEs in vivo.

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## Introduction

In man, most serious occupational diseases arising from environmental chemicals are caused by inhalation of these compounds. With the exception of gases, it might be possible to investigate biological effects of these substances also by oral, intraperitoneal, intravenous or subcutaneous application to experimental animals. However, it is preferable to expose the animals with the compounds in form of gas, aerosols or particles in order to estimate the potential mutagenic risk of chemicals inhalated by man.

In the field of toxicology including cancer research inhalation toxicity has been performed for decades (Gage, 1970). In mutation research especially with mammals, experiments with inhalation mutagenicity are rare and methodical experiments to analyse SCEs in animals exposed to gases are lacking.

As part of investigations on the mutagenic effects in mammals in vivo after subacute inhalation of environmental chemicals, the applicability of the SCE test and the analysis of structural chromosome aberrations were investigated. The chosen test compound was Vinyl chloride, known to be mutagenic and cancerogenic in mammals and man (for review see Bartsch and Montesano, 1975).

## Material and Methods

*Animals.* Adult (10–15 weeks old) Chinese hamsters (*Cricetulus griseus*), weighing 30 g, were used. In all experiments the ratio of female to male hamsters was 1 : 1. During the exposure period the animals were allowed access to food and water.

*Test Compound.* Vinyl chloride (Chlorethylene, $C_2H_3Cl$) was purchased from Merck-Schuchardt, Darmstadt, FRG. The gas was supplied via a two stage pressure regulator and a flow meter in order to measure the flow rate in l/min. The gas was mixed in a mixing chamber with air in different amounts, which was metered from the compressed air taps in the laboratory via a pressure reducing valve and an air flow meter.

*Experimental Design (Fig. 1).* 1.25%, 2.5% or 5% (v/v) VC in air was conveyed through an inhalation chamber, made of Plexiglas®, with the size of 20 × 20 × 20 cm. The fresh gas inlet was on the bottom of the chamber, the outlet was at the lid of the box, diagonal opposite to the inlet. The continuing flow of the gas

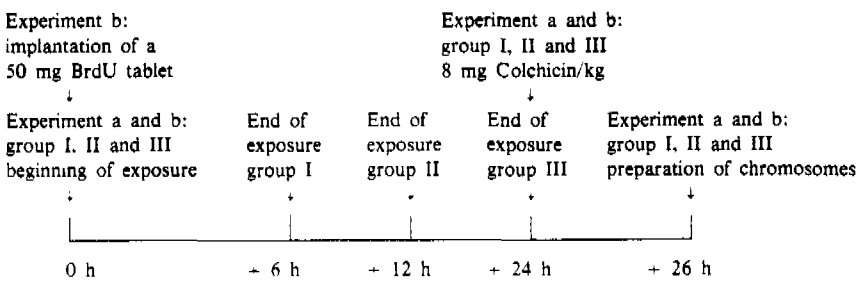


Fig. 1. Experimental design. Analysis of Vinyl chloride induced structural chromosome aberrations (a) and SCEs (b) in bone marrow of Chinese hamsters

mixture was 4 l/min. Up to 4 hamsters (only one sex at a time) were housed in the inhalation chamber. Animals of the negative controls were kept under similar conditions. They were gased with a flow of air only.

*Analysis of Structural Chromosome Aberrrations.* Chinese hamsters were exposed to 2.5% VC in air for 6, 12 or 24 h. Another group was gased with 5% VC for 24 h only. The hamsters received an intraperitoneal injection of 8 mg colchicine/kg body weight 24 h after the beginning of exposure. Bone marrow chromosomes were prepared 2 h later according to Boller and Schmid (1970) and analysed for gaps, breaks, fragments, deletions and exchanges. Metaphases with 5 and more aberrations were listed as multiple aberrations.

*Analysis of Induced SCEs.* Chinese hamsters were exposed to VC for 6, 12 or 24 h. The VC concentrations were either 1.25% or 2.5%. The BrdU-tablet method (Allen et al., 1977) was used to obtain in vivo induced sister chromatid exchanges. Therefore a 50 mg BrdU-tablet (Boehringer, Mannheim, FRG) was implanted subcutaneously into the neck of 30 g weighing Chinese hamsters immediately before starting the VC exposure. Colchicine was injected 24 h later, bone marrow chromosomes were prepared another 2 h later. After an air drying of 2 to 3 days the chromosomes were stained with Bisbenzimide (Hoechst 33258) dye (Riedel de Haen, Seelze-Hannover, FRG) and Giemsa according to Epplen et al. (1975).

*Statistics.* The data of the SCE test have been statistically analysed using the $t$-test. The significance of differences in the yield of metaphases with aberrations was verified using the $X^2$-test.

## Results

The dose levels for VC were selected on the basis of toxicity studies. Groups of 4 hamsters were exposed for 24 h to VC in air at 1.25%, 2.5%, 5% and 7.5%. 5% VC was chosen for the mutagenicity experiments as the highest exposure level as this was found to be the maximum tolerated dose. No mortality occurs in this group within the 24 hourly experiment, whereas higher doses were toxic in all cases.

The number and percentage of metaphases with structural chromosome aberrations are shown in Table 1. In 1400 metaphases of 14 hamsters of the control group only 1 break (0.1%) and 8 gaps were found. The percentage of metaphases with

**Table 1.** Chromosome aberrations in bone marrow cells of Chinese hamster

| Dose and time of exposure | No. of animals | No. of metaphases | Metaphases with aberrations | | | | Metaphases with | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Gaps included | | Gaps excluded | | G | B+F | D | E | m.a. |
| | | | No. | % | No. | % | | | | | |
| Control | 14 | 1400 | 9 | 0.6 | 1 | 0.1 | 8 | 1 | 0 | 0 | 0 |
| 2.5% VC: 6 h | 4 | 400 | 3 | 0.8 | 3 | 0.8[a] | 0 | 3 | 0 | 0 | 0 |
| 2.5% VC: 12 h | 4 | 400 | 3 | 0.8 | 3 | 0.8[a] | 0 | 3 | 0 | 0 | 0 |
| 2.5% VC: 24 h | 4 | 400 | 29 | 7.3[a] | 18 | 4.5[a] | 11 | 18 | 0 | 0 | 0 |
| 5.0% VC: 24 h | 10 | 1000 | 257 | 25.7[a] | 225 | 22.5[a] | 32 | 87 | 9 | 76 | 53 |

G gaps, B + F breaks a. fragments, D deletions, E exchanges, m.a. multiple aberrations
[a] $p < 0.02$

**Table 2.** In vivo induced SCEs in bone marrow cells of Chinese hamster

| Time of exposure | No. of animals | No. of meta-phases | 1.25% VC in air SCEs/cell ± SD | No. of animals | No. of meta-phases | 2.5% VC in air SCEs/cell ± SD |
|---|---|---|---|---|---|---|
| Control | 8 | 400 | 4.41 ± 0.58 | 8 | 400 | 4.56 ± 0.92 |
| 6 h | 4 | 200 | 8.72[a] ± 1.36 | 4 | 200 | 12.66[a] ± 3.72 |
| 12 h | 4 | 200 | 17.51[a] ± 1.55 | 4 | 200 | 19.80[a] ± 2.60 |
| 24 h | 4 | 200 | 22.33[a] ± 0.67 | 4 | 200 | 33.25[a] ± 3.26 |

[a] $p < 0.02$

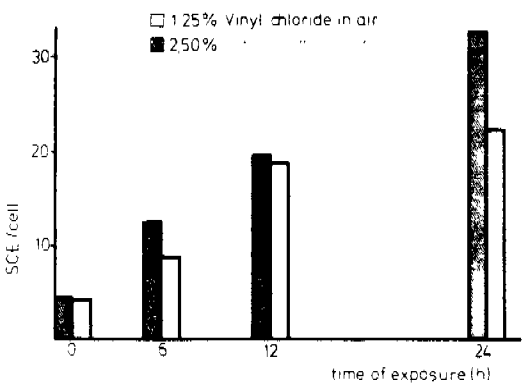


**Fig. 2.** In vivo induced sister chromatid exchanges in bone marrow chromosomes of Chinese hamster

aberrations was slightly increased in those hamsters, exposed to 2.5% VC for 6 h. Similar results were obtained following an exposure time of 24 h. In both groups the values for induced aberrations (only breaks and fragments) were 0.8%. A distinct increase was first noted following an inhalation time of 24 h. In 4.5% of the analysed metaphases breaks and fragments were induced. Gaps included, the percentage of affected metaphases increased from 0.6% in the controls to 7.3%. Even 22.5% aberrant metaphases (25.7% including gaps) were found if the dose of the 24 h exposure was increased to 5%. The majority of aberrations were breaks and fragments, followed by exchanges, multiple aberrations, gaps and deletions.

The number of in vivo induced SCEs (Table 2) depends – as demonstrated for structural chromosome aberrations – both on dose and length of exposure to VC. The 6 h lasting inhalation of 1.25% VC even doubled the SCE frequency. 4.41 SCEs per cell were counted in the controls, whereas 8.72 were detected in this experimental group. A lengthening of the inhalation time increased the number of SCEs. 17.51 SCEs per cell were found following 12 h lasting inhalation and 22.33 after an exposure time of 24 h. After application of 2.5% the SCE frequency increased in all exposure-time-groups as compared to the dose of 1.25%: 12.66 (6 h of exposure), 19.80 (12 h) and at maximum 33.25 SCEs per cell (24 h) were analysed. The increase was nearly linear (Fig. 2) at the investigated dose range.

## Discussion

Several studies on human lymphocytes of workers exposed to VC have been performed to ascertain whether mutations were induced in somatic tissues:

Kučerová et al. (1979) reported both, an increase of aberrations and SCEs in workers exposed for 10–27 years to mean annual doses of VC about 20–150 ppm of air. The SCE rates observed in this study (increase from 9.41 to 13.80 SCEs per cell), however, have to be considered with reservation. Four out of the examined persons were smokers and 8 of them had more or less drinking habits and both of these habits are supposed to be mutagenic. The average number of SCEs for example is 17.2 in heavy smokers, compared to 13.2 in nonsmokers (Lambert et al., 1978).

The level of chromosome aberrations was also positively correlated to the duration of employment, as demonstrated by Purchase et al. (1978). The different findings of Funes-Cravioto et al. (1975) may be the result of other factors, such as job category. In a study of Purchase et al. (1978), for example, the autoclave workers who operated and cleaned the autoclaves, had the greatest number of aberrations of all plant workers. No increase was observed in workers who were not involved in the polymerisation process (Kilian et al., 1975).

Correlations between the VC concentrations at work place and induced mutations were reported also by Hansteen et al. (1978). Cytogenetic studies were performed first in 1974 on workers who had been heavily exposed to VC for years. The study was repeated 2 years later with the same workers. Meanwhile the concentrations in plants were reduced and the workers had only a low exposure to VC (< 1 ppm). Chromosome aberrations increased compared to unexposed workers in the 1974 study, whereas in the follow-up study, the chromosome breakage frequency in these workers decreased to control level. The SCEs were tested in 1977 only, and no difference was found between workers and controls.

In heavily exposed workers, resulting in symptoms of VC illness such as acroosteolysis, Raynaud syndrome, thrombocytopenia and liver disturbances, the rate of aberrations increased, compared to unexposed persons, as well as to VC workers without these syndroms (Fleig and Thiess, 1978).

Comparing the history of exposure, it becomes evident that the yield of VC-induced chromosome aberrations in lymphocytes of workers depend on different factors: the category of workplace correlated to the VC concentration in the PVC plant and the length of exposure.

Besides the induction of chromosome aberrations in somatic tissues mutational events in germ cells and the transmission to the following generations were discussed by Infante et al. (1976). They confirmed a significant excess of fetal mortality among wives of workers, who were occupationally exposed to VC. The data however, have been criticised (Paddle, 1976) because of inaccuracies in statistical methods.

Experimental investigations with mammals did not support the findings in man. Anderson et al. (1976) exposed mice to VC by inhalation up to 30000 ppm for 6 h a day for 5 days. No mutagenic effects on any maturation stage of spermatogenesis in treated males were detected as measured by the dominant lethal test. Negative results were obtained also in drosophila when tests on dominant lethals and translocations were carried out with VC at 30000 ppm for 2 days. It was mutagenic only in the recessive lethal test (Verburgt and Vogel, 1977). The conclusion that chromosome breakage is not

a reliable measure of mutagenic activity of VC (Verburgt and Vogel, 1977) might be relevant for the test animal drosophila, not however for mammals, as shown by Fleig and Thiess (1978a, b).

Induced aberrations were provable in Chinese hamster bone marrow chromosomes when treated by inhalation up to 5000 ppm VC for 4 h a day for 5 days (Fleig, 1977; Fleig and Thiess 1978b). In contrast to our finding with higher doses no dose-effect-relationships were obtained.

In the present experiments a distinct increase was measured following an exposure to 2.5% VC. Considering the average concentrations of VC at the work place of PVC plants in the years 1950–1954 were 2000 ppm (Hansteen et al., 1978), the chosen dose levels in our experiments were approximately 60 to 250 times higher. We have to state, however, that chromosome aberrations were induced following an exposure to inhalation of only 6 to 24 h.

The SCE test in vivo is increasingly used in the field of mutagenicity testing because of the high sensitivity. Performing the primary method (Vogel and Bauknecht, 1976; Allen and Latt, 1976) BrdU had to be injected every hour for 6 to 8 h. Today using the BrdU tablet method (Allen et al., 1977), BrdU is subcutaneously implanted in form of a 50 mg tablet into the neck of Chinese hamster and chromosomes are prepared 26 h later. This technique enables to expose test animals by inhalation immediately after the tablet implantation without interruption up to 24 h when colchicine is injected. The applicability of this method is demonstrated in the experiments presented here. The SCE frequency was even doubled following an exposure to 1.25% VC for only 6 h and increased with increasing doses of VC and duration of exposure.

As mentioned above, the VC concentration at the work place was reduced within the last years. Clues, whether the present technical guiding concentration of 5 ppm (Deutsche Forschungsgemeinschaft, 1979) is a tolerable dose, cannot be drawn from these experiments. As demonstrated, the exposure time plays a considerable part in the yeald of induced mutations. A long term exposure for years, the situation for man at the work place, cannot be reproduced experimentally. The establishment of maximum tolerable values, however, was not the aim of this study. We intended to demonstrate that both cytogenetic methods in vivo are applicable to evaluate a possible mutagenic risk of environmental agents in gaseous state. For this reason, as usually in the field of mutagenicity testing, the animals were acutely exposed to the test compound with dose levels selected on the basis of toxicity studies. On the basis of this experimental design, the SCE test as well as the analysis of chromosome aberrations lead to clear cut dose response relations.

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## References

Allen, J. W., Latt, S. A.: Analysis of sister chromatid exchange formation in vivo in mouse spermatogonia as a new test system for environmental mutagens. Nature **260**, 449–451 (1976)

Allen, J. W., Shuler, C. F., Mendes, R. W., Latt, S. A.: A simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromodeoxyuridine tablets. Cytogenet. Cell Genet. **18**, 231–237 (1977)

Anderson, D., Hodge, M. C. E., Purchase, I. F. H.: Vinyl chloride: Dominant lethal studies in male CD-1 mice. Mutat. Res. **40**, 359–370 (1976)

Bartsch, H., Montesano, R.: Mutagenic and carcinogenic effects of vinyl chloride. Mutat. Res. **32**, 93–114 (1975)

Boller, K., Schmid, W.: Chemische Mutagenese beim Säuger. Humangenetik **11**, 35–54 (1970)

Deutsche Forschungsgemeinschaft: Maximum concentrations at the workplace 1979. Commission for investigation of health hazards of chemical compounds in the work area. Report No. XV. Boppard: Harald Boldt Verlag 1979

Epplen, J. T., Siebers, J. W., Vogel, W.: DNA replication patterns of human chromosomes from fibroblasts and amniotic fluid cells revealed by a Giemsa staining technique. Cytogenet. Cell Genet. **15**, 177–185 (1975)

Fleig, I.: Zur Mutagenität von Vinylchlorid. Untersuchungen an Säuger und Mensch. Dissertation, Ruprecht-Karl-Universität, Heidelberg, 1977

Fleig, I., Thiess, A. M.: External chromosome studies undertaken on persons and animals with VC illness. Mutat. Res. **53**, 187 (1978a)

Fleig, I., Thiess, A. M.: Mutagenicity of vinyl chloride. J. Occup. Med. **20**, 557–561 (1978b)

Funes-Cravioto, F., Lambert, B., Lindsten, J., Ehrenberg, L., Natarajan, A. T., Osterman-Golkar, S.: Chromosome aberrations in workers exposed to vinyl chloride. Lancet **1**, 459 (**1975**)

Gage, J. C.: The subacute inhalation toxicity of 109 industrial chemicals. Br. J. Ind. Med. **27**, 1–18 (1970)

Hansteen, I.-L., Hillestad, L., Thiis-Evensen, E., Storetvedt Heldaas, S.: Effects of vinyl chloride in man. A cytogenetic follow-up study. Mutat. Res. **51**, 271–278 (1978)

Infante, P. F., Wagoner, J. K., Waxweiler, R. J.: Carcinogenic, mutagenic and teratogenic risks associated with vinyl chloride. Mutat. Res. **41**, 131–142 (1976)

Kilian, D. J., Picciano, D. J., Jacobson, C. B.: Industrial monitoring: A cytogenetic approach. Ann. NY Acad. Sci. **269**, 4–11 (1975)

Kučerová, M., Polivková, Z., Bátora, J.: Comparative evaluation of the frequency of chromosomal aberrations and the SCE numbers in peripheral lymphocytes of workers occupationally exposed to vinyl chloride monomer. Mutat. Res. **67**, 97–100 (1979)

Lambert, B., Lindblad, A., Nordenskyjöld, M., Werelius, B.: Increased frequency of sister chromatid exchanges in cigarette smokers. Hereditas **88**, 147–149 (1978)

Paddle, G. M.: Genetic risks of vinyl chloride. Lancet **1**, 1079 (**1976**)

Purchase, I. F. H., Richardson, C. R., Anderson, D., Paddle, G. M., Adams, W. G. F.: Chromosomal analyses in vinyl chloride-exposed workers. Mutat. Res. **57**, 325–334 (1978)

Verburgt, F. G., Vogel, E.: Vinyl chloride mutagenesis in *Drosophila Melanogaster*. Mutat. Res. **48**, 327–336 (1977)

Vogel, W., Bauknecht, T.: Differential chromatid staining by in vivo treatment as a mutagenicity test system. Nature **260**, 448–449 (1976)

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