Vinyl chloride

Vinyl Chloride Cytogenetics

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This report presents cytogenetic findings from a group of 209 workers employed for up to 28 years in the manufacture of vinyl chloride monomer at the Texas Division of Dow Chemical U.S.A. Cytogenetic evaluation results from this group were compared to results found in examination of individuals being considered for employment. Statistical analyses were performed on a group basis for chromatid aberrations, chromosome aberrations and proportion of abnormal cells; no statistical difference of significance was found between the two groups. Comparison of these results with reported studies suggests that the level of cytogenetic aberrations in vinyl chloride workers is probably related to the length and level of exposure, and that risk of adverse genetic effect can be avoided in cohtrolled, minimal-exposure environments.

Vinyl chloride (VC) has been reported to be carcinogenic in animals[1,2] and human beings[3] and mutagenic in bacterial test systems.[4,5,6] There have been reports of increased frequencies of chromosomal aberrations in workers exposed to vinyl chloride at polyvinyl chloride (PVC) facilities[7,8,9] Similar increases were not found upon cytogenetic evaluation of German PVC workers[10] nor in our own preliminary investigation of a group of American VC workers.[11] One study based on interviews with male employees has indicated an increased rate of fetal wastage among wives of VC-exposed workmen involved in polymerization operations.[12]

We have recently completed cytogenetic evaluation of all currently-employed workers exposed to vinyl chloride at the Dow (Freeport) Texas Division. Both vinyl chloride and vinylidene chloride are produced in this plant. Vinylidene chloride has also been reported as mutagenic in bacterial test systems.[13] However, while these Dow workers are involved with the production of both compounds, the degree of contact with vinylidene chloride is far less than the exposure to vinyl chloride.

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Vinyl chloride monomer has been produced at the Texas Division of Dow Chemical U.S.A. since 1948. There are no vinyl chloride polymerization operations. Average exposure levels, as is the case for almost all vinyl chloride monomer plants, have been generally lower than those reported for facilities involved in polymerization operations.[14] Although the threshold limit values for vinyl chloride had been 500 parts per million (ppm) until 1974, Dow had established a goal of 50 ppm or below in 1959.[15] The current Occupational Health and Safety Administration (OSHA) standard for vinyl chloride is one ppm as a time-weighted-average over an eight-hour workday. We have found that the TWA concentration of vinyl chloride for all VC-related job classifications was approximately 5 ppm from 1968 to 1973; in 1974, TWA concentrations were 1 to 2 ppm. Since 1975, average levels of exposure have been lowered to less than 1 ppm. Estimates of average exposure levels for the years prior to 1968 are less reliable. For the purposes of this study, estimates of exposure were calculated for specific job classifications, based on records of both personnel and area monitoring (Table 1). However, actual exposure may vary from individual to individual within the same job classification and accidental, short-term exposures of some workers to concentrations in excess of the standard probably occur from time to time. Documentation of such incidents is difficult.

Methods

Our study group was composed of 209 workers who had worked in the vinyl chloride plant for periods ranging between one and 332 months (average, 48.3 months) at the time of this investigation. As part of the medical surveillance program for this group, peripheral blood samples were obtained, and evaluation of lymphocyte chromosomes was performed. Lymphocytes were cultured using a modification of the Moorhead technique.[16] Standard procedures, as previously described,[17] were used for incubation, processing, and analysis.

Findings were compared to cytogenetic data from a group of 295 "preemployment examinees" who had chromosome evaluation done as part of routine preemployment examination and who, at that time, had no known exposure to chromosome-break-

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Table 1. — Estimated Exposure to Vinyl Chloride (VC) for Vinyl Chloride-Related Job Classifications.

Job Classification	Estimated Exposure in Parts per Million, as Time-Weighted-Average 1973-1974	1960-1972	Before 1960
Supervisor	1.7	4.4	8.2
R&D Engineer	1.7	4.4	8.2
Production Engineer	1.7	4.4	8.2
Technical Specialist	0.5	0.5	0.5
Development Lab (Lab Supv.)	1.2	3.9	7.7
Quality Control Lab	8.7	11.4	15.2
Instrument Man	1.3	4.0	7.8
Electrician	1.3	4.0	7.8
Coverers (Insulators)	0.3	0.3	0.3
Painter	0.7	0.7	0.7
Pipefitter	0.9	0.9	0.9
Machinist	2.4	5.1	8.9
Welder (Boilermaker)	0.6	0.6	0.6
Maintenance (Utility Man)	1.3	4.0	7.8
Material Handler	0.5	0.5	0.5
Production Foreman	1.3	4.0	7.8
Shift Foreman	4.4	7.1	10.9
Control A Op. (V2 & Chloride)	5.2	7.9	11.7
Control A Op. (E & CI)	0.9	0.9	0.9
Control A Op. (Vinyl)	2.8	5.5	9.3
Control A Op. (Chlorination)	2.3	5.0	8.0
Control B Op. (Oxy & Chloride)	0.7	0.7	0.7
Control C Op. (Chloride & Vinyl)	4.6	7.3	11.1
Control C Op. (Chlorination)	2.6	5.3	9.1
Class 1 Op.	5.3	8.0	11.8
Class 1 Op. (Parts)	0.5	0.5	0.5
Class 3 Op.	2.6	5.3	9.1
Production Clerk	0.7	0.7	0.7
Unit Manager	0.7	0.7	0.7

ing agents. The records selected for inclusion in the control group were matched to those of the study group, insofar as possible, for sex, number of cells analyzed, and time period during which the culture was initiated.

Age variation between the two groups could not be completely eliminated: the average age of the vinyl chloride workers was 39.5 years (range, 18 to 67 years), and the average age of the preemployment control group was 25.1 years (range 18 to 50 years).

Results

As shown in Table 2, data from both groups — vinyl chloride workers and preemployment controls — were scored and compared on the basis of chromatid breaks, chromosome breaks; rings, dicentrics, and exchange figures; and the proportion of abnormal cells. The proportion of abnormal cells was calculated to show the overall frequency of aberrant cells, that is, the ratio of cells with at least one aberration to the total number of cells examined. Results, expressed in terms of the mean percentage for these categories of aberration, showed no major differences between the two groups for any of the classifications.

Using Chi-square analysis, it was decided to see if differences in various aberration rates could be detected within the two groups. Accordingly, both groups were divided into those showing zero-to-five percent aberrations and those showing greater-than-five

Table 2. — Cytogenetic Study of 209 Workers Exposed to Vinyl Chloride.

	Workers	Controls
No. of cultures	209	295
No. of cells	10,483	14,761
Chromatid breaks	2.4%	3.6%
Chromosome breaks	1.0%	1.1%
Rings, dicentrics, and exchanges	0.4%	0.2%
Abnormal cells	3.7%	4.5%

percent aberrations, and the vinyl chloride workers were separated into those with estimated TWA exposure levels of less-than-1 ppm, from 1-to-5 ppm, and greater-than-5 ppm. The estimated exposure levels were based on calculations for specific job classifications to which members of the vinyl chloride study group were assigned. Three categories of aberration were chosen for this analysis: chromatid breaks, chromosome breaks, and the proportion of abnormal cells.

As shown in Table 3, no significant differences were found when these groupings were compared on the basis of chromatid aberrations. Results from those workers with estimated exposures of greater than 5 parts per million are almost identical to those of the control group.

Similar conclusions were reached upon evaluation of the data for chromosome breaks (Table 4) and the proportion of abnormal cells (Table 5). In all three cases, most of both groups were found to have zero-to-five percent aberrations, and the vinyl chloride workers with the highest level of estimated exposure showed aberration rates not significantly different than those of the control group. Findings for both groups, workers and controls, are considered to be within the range of normal variation as seen in this laboratory.

Discussion

The reports that have appeared, to date, concerning chromosome aberrations in vinyl chloride workers have not been in agreement. This conflict may be due either to the small number of workers studied or to differences in exposure levels, or both. The Swedish group studied by Funes-Cravioto et al[18] was composed of seven PVC workers who had been exposed for nine to 29 years and who were found to have an increased frequency of chromosome breakage. The level of exposure for this group was

Table 3. — Distribution of Chromatid Aberrations Related to Vinyl Chloride (VC) Exposure.

	No. in Group	% of Group with 0-5% Aberrations	% of Group with >5% Aberrations
Exposure to VC*	209		
<1 ppm	70	90%	10%
1-5 ppm	98	77	23
>5 ppm	41	80	20
Controls	295	75	25

$\chi^2_{(3)} = 7.75$ ($P \cong 0.06$)

* Exposure levels are estimates based on calculations for specific job classifications; prior exposure for individuals may have been higher or lower; exposure levels for individuals are known to vary within job classifications.

Table 4. — Distribution of Chromosome Aberrations Related to Vinyl Chloride (VC) Exposure.

	No. in Group	% of Group with 0-5% Aberrations	% of Group with >5% Aberrations
Exposure to VC	209		
<1 ppm	70	96%	4%
1-5 ppm	98	94	6
>5 ppm	41	95	5
Controls	295	94	6

$\chi^2(3) = 0.355$ ($P = 0.95$)

Table 5. — Distribution of Abnormal Cells Related to Vinyl Chloride (VC) Exposure.

	No. in Group	% of Group with 0-5% Aberrations	% of Group with >5% Aberrations
Exposure to VC	209		
<1 ppm	70	84%	16%
1-5 ppm	98	71	29
>5 ppm	41	73	27
Controls	295	70	30

$\chi^2(3) = 5.97$ ($P = 0.12$)

reported to have continuously decreased over a period of years until immediately prior to the study when VC concentrations in the polymerization department were estimated to be 20 to 30 ppm

The U.S. group of 11 PVC workers surveyed by Ducatman et al[19] was also reported to show an increased rate of chromosome breakage following exposures estimated to have been in excess of 500 ppm at times. The group of ten German workers investigated by Fleig and Thiess[20] worked with either VC or PVC or both for periods ranging between six and 34 years and with exposures estimated to have decreased from greater than 500 ppm in 1945 to between 10 and 25 ppm in 1974. Evaluation of this group for increased aberration rates was negative.

The study of British PVC workers by Purchase *et al.*[21] concluded that the frequency of cytogenetic aberrations was increased in 56 exposed workers as compared to 24 nonexposed individuals. Estimates of the levels of exposure were not given in this report. The medical director of the surveyed group, however, has informed us that while further analyses of the data confirmed the findings of increased aberrations in workers exposed to "higher" levels of vinyl chloride, no differences between worker and control groups were detected upon evaluation of the data for workers exposed to "lower" levels.

An earlier report from our laboratory[22] concluded that there were no cytogenetic differences of statistical significance between a group of 121 workers exposed to vinyl chloride and a 75-person control group. As in the work reported here, there was a discrepancy in the age composition of the two groups, because applicants seeking employment tend to be younger than those already settled into jobs. We do not believe, however, that this difference is a confounding factor in our comparison since the difference is not great and because it has been shown[23] that the cytogenetic change most often associated with aging is chromosome loss, rather than chromosome breakage.

On the basis of our negative findings and the conflicting findings reported by others, we believe that the level of chromosome aberrations in workers exposed to vinyl chloride is probably related to the length and level of exposure and that the risk of adverse cytogenetic effects can be avoided in controlled, minimal-exposure environments Cytogenetic dose-response curves, similar to that suggested here, have been reported for x-irradiated ankylosing spondylitics,[24] A-bomb survivors,[25] radium dial painters,[26] persons exposed to Thorotrast,[27] and workers exposed to lead [28] It has also been noted that the groups exposed to radiation later demonstrated significant increases in neoplastic incidence. A-bomb survivors, for example, have shown increased rates of leukemia and thyroid carcinoma;[29] radium dial painters were found to be at increased risk of osteogenic sarcoma;[30] and thorium dioxide-exposed persons have an increased rate of liver tumors.[31] This relationship between chromosomal breakage and neoplasia strongly suggests that cytogenetic analyses may be a useful tool for detecting environmental situations which may be associated with increased cancer risks to the workers

The present study is one of several involving Texas plant employees to investigate the possibility that increased rates of fetal loss or birth defects in offspring are experienced by wives of vinyl chloride workmen and to determine the morbidity-mortality experience of all past and present VC workers. A search has been made for cases of angiosarcoma of the liver; none have been found in our study group (533 individuals) which includes all past and present VC workers. Current workers are also being monitored as part of the continuing medical surveillance program.

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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

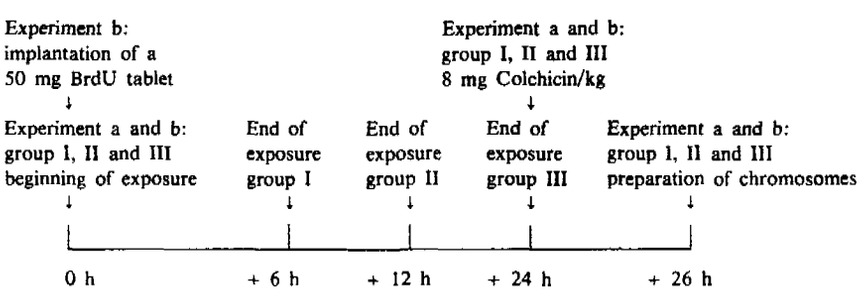

Fig. 1. Experimental design. Analysis of Vinyl chloride induced structural chromosome aberrations (a) and SCEs (b) in bone marrow of Chinese hamsters

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 metaphases	1.25% VC in air SCEs/cell ± SD	No. of animals	No. of metaphases	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] $p < 0.02$

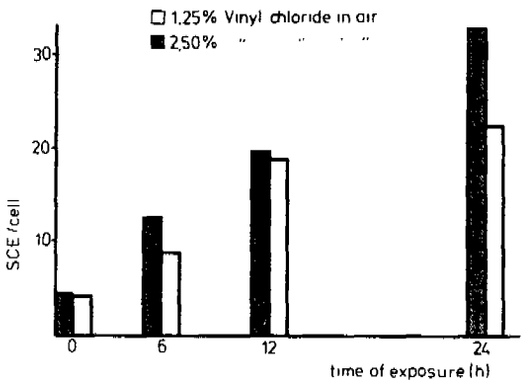

Fig. 2. In vivo induced sister chromatid exchanges in bone marrow chromosomes of Chinese hamster

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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