Seminar in Occupational Medicine

Mutagenicity of Vinyl Chloride

External Chromosome Studies on Persons With and Without VC Illness, and on VC Exposed Animals

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Review of the Literature

According to our knowledge, nine studies[3-10 13] relating to vinyl chloride (VC) problems have been undertaken. However, no conformity could be reached from these results. As Table 1 shows, five studies had a higher rate of chromosome aberration in the VC exposed group than in the control group. On the other hand, four further studies produced negative results. All those investigations made on animal and sub-mammalian species[1 11-14] are shown in Fig 1.

Personal Investigations

Investigations on persons concerned with the manufacture of VC/PVC with estimated exposure have been undertaken in our medical department.[5] In this group we cannot make any definite statements regarding the rate of exposure, but it is most probable that the assumed values taken from abroad are also applicable to the Federal Republic of Germany.[15] These values are: 1945-1950 — 1,000 ppm; 1950-1960 — 400-500 ppm; 1960-1970 — 200-400 ppm; and 1971-1973 — below 150 ppm.

In addition, investigations were undertaken on persons in the VC/PVC processing plants who have undergone monitored VC exposure (Fig 2).[5] Both groups were combined as "Exposed persons showing no symptoms of VC illness" (Table 3 and Fig 3).

Before commencing the study, anamnestic data were collected regarding sex, age, occupational exposure(s), medical x-ray treatment either for diagnostic or therapeutic purposes, recent viral diseases and vaccinations, consumption of drugs, and smoking habits, in order to discard those subjects who have multiple exposures. We matched these subjects with healthy controls of the same sex (in this case male) and approximately the same age who

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Table 1. — A Survey of Results from VC Exposed Persons.

Authors	No. of Probands	Length of Exposure (Years)	% Chromosome Aberrations VC Exposed (Average)	% Chromosome Aberrations Controls	Mutagenic Effect	Pathological Results (VC Syndrome)
Fleig, Thiess (1974)	10 workers	4-26	7.5	5.5	Negative	No pathological results
Ducatman, Hirschhorn Selikoff (1975)	11 workers 10 controls	4-28	9.82	6.4	Positive	—
Funes-Cravioto et al (1975)	7 workers 3 controls	9-29	9.52	1.94	Positive	1 thrombocytopenia
Hansteen, Hillestad	39 workers					
Thiis-Evenson (1975)	16 controls	—	3.41	1.79	Positive	—
Kilian, Picciano (1975)	203 workers 108 controls	Up to 30	3.9	5.5	Negative	—
Lange Schwinger, Veltman (1975)	12 workers	3.25-21	—	—	Negative	Thrombocytopenia, splenomegaly acroosteolysis, Raynaud's syndrome scleroderma
Purchase, Richardson Anderson (1975)	56 workers 24 controls	—	8.13	4.18	Positive	—
Fleig (1977)	20 workers 20 controls	4.30	11.2	5.5	Positive	Thrombocytopenia splenomegaly scleroderma Raynaud's syndrome liver fibrosis, esophagus, and stomach varices of the fundus, acroosteolysis
Leonard et al (1977)	18 workers 10 controls	2.17	1.9 resp 2.8	2.6	More severe chromosome anomalies — due to radiograph?	—

Authors	Test – Method	Exposure	Mutagenic Effect	Aberration Type
Bartsch Malaveille Montesano (1975)	a) AMES-Test TA 1530, TA 1535, G 46 b) AMES-Test TA 1536, TA 1537, TA 1538	2,000, 20,000, und 200 000 ppm 6 h and 48 h	a) positive b) negative	Point mutations
Rannug Johannson Ramel Wachtmeister (74)	a) AMES-Test TA 1535 b) AMES-Test TA 1536, TA 1537, TA 1538	200,000 ppm 30, 60, 90 Mins.	a) positive b) negative	Point mutations
Laprianо et al (1976)	a) AMES-Test Schizosaccharomyces pombe b) AMES-Test Saccharomyces cerevisae c) HOST-MEDIATED-ASSAY (Mouse)	a) 3,000 ppm b) 3,000 ppm c) 700 mg/kg oral	a) positive b) positive c) positive	Point mutations
Magnusson Ramel (1975)	MULLER-5-Method (Drosophila)	not known	positive	Recessive lethal mutations
Purchase Richardson Anderson (1975)	DOMINANT-LETHAL-Test (Mouse)	3,000 10,000 and 30,000 ppm	negative	No dominant lethal mutations
Reig (1977)	BONE MARROW (Hamster)	2,500, 5,000 ppm	positive	Chromosome aberrations

Fig 1. — A survey of the results using VC in various tests.

Recording Point	VC content in ppm	Remarks
Fluid Mixer	< 1	At headlevel in the position occupied by the operator
Fluid Mixer Exit	< 1	At headlevel in the position occupied by the operator (after the mixing process.)
Mixing-mill	< 1	At headlevel in the position occupied by the operator
Mixing-mill directly above the nip	12	Position not occupied by personnel
Colander feeding point	< 1	At headlevel in the position occupied by the operator
Colander take-off	< 1	At headlevel in the position occupied by the operator
Extruder jet	< 1–4	Only temporarily occupied by the operator
Extruder feed-hopper	< 1–28	Only temporarily occupied by the operator

Fig 2. — Recordings of VC concentrations in the atmosphere in the region of the KAE Technical Department.

wer not exposed to VC or to any other known chromosome damaging agent but worked in the same factory in different departments (e.g., clerical staff from the sales or technical department). The same questionnaire used for obtaining data on experimental subjects was also used for controls, and the same criteria for exclusion from the study was applied.

Lymphocyte cultures were prepared from both groups and finally processed using the so-called "micro-method." One hundred metaphases per person were investigated, making a total of 3,000, which were analyzed for structural chromosome anomalies. The frequency of aberrant metaphases totaled 3.1% excluding gaps and 7.5% including gaps. The corresponding values in the control group were 2.1% and 5.5%, respectively. The individual datas are depicted in Tables 2 and 3. There was no significant difference in the rate of chromosome aberrations compared with the control group (Fig 3).

Investigations were also made on workers not employed in the BASF but with VC symptoms after an unknown rate f exposure. Within the framework of a research program from the insurance association of the chemical industry and the Verband Kunststofferzeugende Industrie (VKE), 20 workers all showing symptoms of VC illness (Fig 4) were studied. Many of the workers examined showed signs of more than one symptom of VC illness. The statistical evaluation revealed a significant difference between the rate of chromosome aberrations in these persons and those in the control group. The frequency of aberrant metaphases in the exposed group was 5.2% excluding gaps and 11.2% including gaps, compared with 2.1% and 5.5%, respectively, in the control group (Fig 5). The control group was the same one mentioned earlier. The individual datas are depicted in Tables 2 and 4.

External investigations were done on a patient with an angiosarcoma due to VC exposure. According to information received

Table 2. — Chromosome Aberration Rate of Controls.

No.	Age	Analyzed Metaphases	% Aberrant Metaphases Incl. Gaps	% Aberrant Metaphases Excl. Gaps
18	42	100	7	3
22	50	100	7	3
26	33	100	1	—
29	39	100	6	2
132	37	100	3	—
149	56	100	7	2
153	46	100	3	—
206	54	100	3	1
207	44	100	5	1
208	64	100	9	7
209	37	100	7	3
210	62	100	9	4
211	38	100	7	2
212	35	100	5	1
213	57	100	2	1
214	42	100	9	4
216	60	100	7	2
217	33	100	4	2
218	52	100	3	2
219	50	100	7	2

Table 3. — Chromosome Aberration Rate of Exposed Persons Showing No Symptoms of VC Illness.

No.	Age	Duration of Exposure (Years)	Analyzed Metaphases	% Aberrant Metaphases Incl. Gaps	% Aberrant Metaphases Excl. Gaps
5	51	11	100	7	1
6	39	7	100	8	2
14	57	20	100	10	2
76	52	25	100	9	3
82	43	15	100	7	3
83	52	20	100	8	5
84	54	20	100	6	4
86	46	4	100	8	4
87	55	26	100	8	5
101	34	6	100	4	2

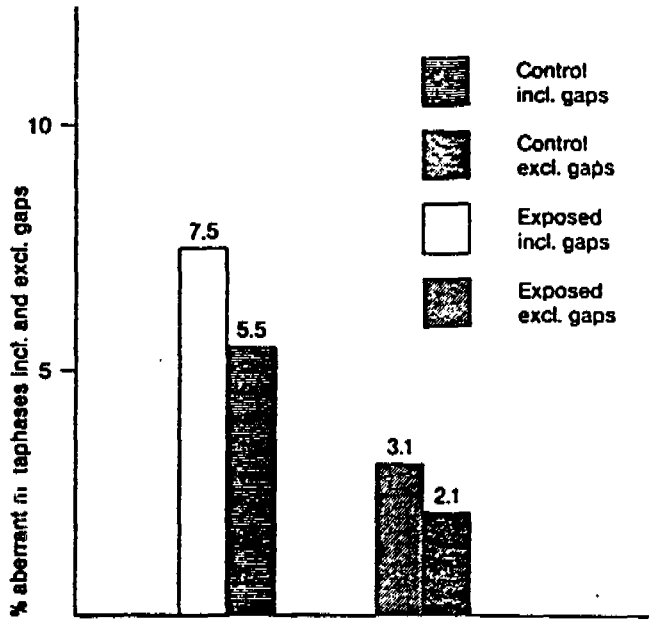

Fig 3. — Chromosome aberration rate of exposed workers showing no symptoms of VC illness.

from the Berufsgenossenschaft. 11 cases from a total of 57 cases of hemangioendothelial sarcoma, attributable to VC/PVC exposure, were discovered in the Federal Republic of Germany (Table 5). One of these 11 persons who is still alive, aged 43, was occupied in the PVC manufacturing area for 13 years. The rate of VC exposure to which he personally was exposed during the last years was between 35 to 150 ppm. He was occupied for 20 to 30 hours per month manually cleaning the autoclaves. In addition, he sometimes took samples from the decontamination plant to the laboratories for testing.

An analysis of 200 metaphases produced a rate of aberrant cells of 7.3% excluding gaps and isogaps, a significant increase compared with a control group which had a rate of 2.1%. The rate of aberrant metaphases including gaps and isogaps was 16.6% compared to the control group, with a rate of 5.5%.

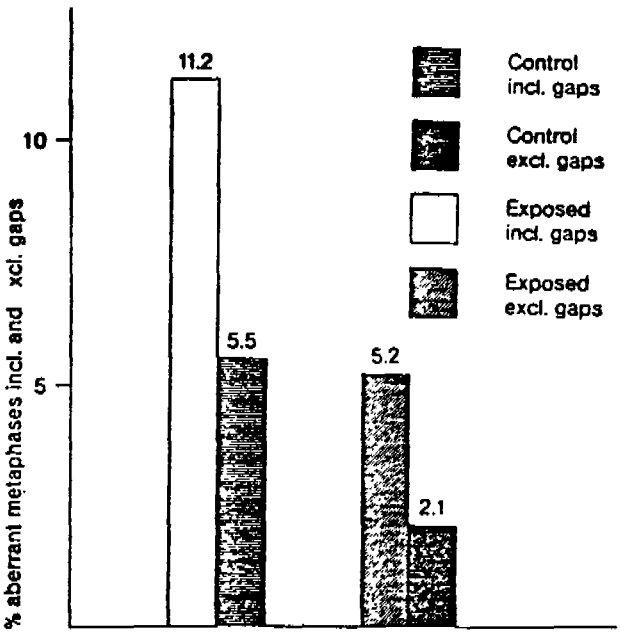

Fig 5. — Chromosome aberration rate of exposed workers showing symptoms of VC illness.

Ref Nr.	Age	Exposure in years	Symptoms	% aberrant incl. gaps	metaph. excl. gaps	Exchanges
90	56	6	Liver fibrosis, oesophagus varices, thrombocytopenia, splenomegaly	10	6	2
91	54	11	Liver fibrosis, portal hypertension, thrombocytopenia, splenomegaly, oesophagus varices	11	6	1
119	33	7	Liver parenchyma damage, thrombocytopenia, splenomegaly	7	2	-
158	37	5	Scleroderma, liver fibrosis, Raynauds syndrome, acroosteolysis, splenomegaly, thrombocytopenia	7	4	1
160	32	6	Liver parenchyma damage, thrombocytopenia	13	7	1
161	42	15	Thrombocytopenia, liver parenchyma damage	10	5	1
162	60	30	Liver parenchyma damage, Raynauds syndrome, thrombocytopenia, oesophagus varices	11	5	-
164	53	19	Liver fibrosis, thrombocytopenia	9	4	1
165	64	22	Liver fibrosis, thrombocytopenia, splenomegaly	11	4	2
167	52	26	Liver parenchyma damage, thrombocytopenia	20	9	1
170	34	4	Liver parenchyma damage, thrombocytopenia, splenomegaly	9	3	1
171	44	9	Liver fibrosis, Raynauds syndrome, acroosteolysis	11	5	1
172	52	5	Liver parenchyma damage, Raynauds syndrome, thrombocytopenia	8	3	1
173	41	5	Liver parenchyma damage, splenomegaly	8	3	1
174	62	10	Liver parenchyma damage, Raynauds syndrome	22	10	2
176	41	16	Liver fibrosis, thrombocytopenia, oesophagus varices	14	6	1
177	38	11	Thrombocytopenia, hepatomegaly	16	8	2
183	49	10	Liver parenchyma damage, splenomegaly	7	5	-
199	52	12	Liver parenchyma damage	8	5	1
203	42	8	Liver fibrosis, splenomegaly	11	5	2

Fig 4. — VC symptoms and rate of chromosome aberration in the exposed probands.

Apart from our investigations on human beings, tests were also performed using bone marrow of Chinese hamsters. Two types of tests were undertaken: (1) inhalation tests with dosages of 2,500 ppm and 5,000 ppm, respectively, for four hours a day, 24 hours apart over a five-day period, and (2) intraperitoneal injections of 300 mg/kg and 600 mg/kg, respectively, given in five injections, 24 hours apart, over a five-day period. Table 6 shows a survey of all important data concerning the arrangement of our animal investigations. Both tests (Figs 6 and 7) showed that the frequency of structural anomalies, inclusive and exclusive of gaps in the bone marrow cells, was significantly higher compared to the control group.[4]

Discussion

The mutagenic effect of vinyl chloride mentioned in various sources of literature, bacterially tested, investigated on mammals, sub-mammals and human beings, has been confirmed by our investigations on mammals (2,500 ppm and 300 mg/kg) and man (5000 ppm and 600 mg/kg).

The most important point to mention is that an increased rate of chromosome aberration could be found only in those persons showing symptoms of VC illness, as well as in an angiosarcoma case. One very important fact must, however, be taken into account. Nine months before blood samples were taken (February 1, 1977), the patient with the angiosarcoma was given polychemotherapy which was comprised of applications of 150 mg Adriblastin, 120 mg Vincristin, 3,000 mg Endoxan, and 6,000

Table 4. — Chromosome Aberration Rate of Exposed Persons Showing Symptoms of VC Illness.

No.	Age	Duration of Exposure (Years)	Analyzed Metaphases	% Aberrant Metaphases Incl. Gaps	Excl. Gaps
90	56	6	100	10	6
91	54	11	100	11	6
119	33	7	100	7	2
158	37	5	100	7	4
160	32	6	100	13	7
161	42	15	100	10	5
162	60	30	100	11	5
164	53	19	100	9	4
165	64	22	100	11	4
167	52	26	100	20	9
170	34	4	100	9	3
171	44	9	100	11	5
172	52	5	100	8	3
173	41	5	100	8	3
174	62	21	100	22	10
176	41	16	100	14	6
177	38	11	100	18	8
183	49	10	100	7	5
199	52	12	100	8	5
203	42	8	100	11	5

mg DTIC (Dacarbazin). It is, therefore, not possible to say whether or not the results we have obtained are solely due to the patient's exposure to VC or whether his chemotherapeutical treatment, together with the exposure rate of VC, affected our results.[2]

Summary

Chromosome analysis was undertaken on lymphocyte cultures taken from (1) six workers with estimated exposure to VC and four workers with monitored exposure to VC (employed in the BASF); (2) 20 workers showing symptoms of VC illness with unknown exposure and one angiosarcoma case, due to VC exposure (not employed in the BASF); and, (3) on bone marrow cells of Chinese hamsters after inhalation of 2,500 ppm or 5,000 ppm, or after intraperitoneal injections of 300 mg/kg or 600 mg/kg. The proband groups showing symptoms of VC illness, the only living angiosarcoma case, and the animal test show a significant increase in the rate of aberrations in comparison to the control group.

Table 6. — Test Procedure.

Animal species	Chinese hamsters
Animal's age	10-14 weeks
Animal's weight	26-30 g
Substance	Vinyl chloride
Test 1	
Type of administration	Inhalation
Interval of administration	24 hours
Dosage	2,500 ppm/5 days/4 hours 5,000 ppm/5 days/4 hours
Time until sacrificed after last administration	6 hours
Test 2	
Type of administration	i.p. injection
Solvent	Olive oil
Interval of administration	24 hours
Quantity administered	5 x 0.5 ml
Dosage	300 mg/kg body weight 600 mg/kg body weight
Time until sacrificed after last administration	6 hours

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Table 5. — Survey of Angiosarcomas of the Liver Due to VC Exposure in the Federal Republic of Germany.

Ref. No.	Plant	Occupation	Date of Birth	Deceased	Age at Diagnosis	Period of Exposure (Years)	Latency Period (Years)
1	4	PVC production autoclever	06/04/30	01/25/69	38	12	1/2
2	4	PVC production autoclever	07/26/31	12/14/71	39	11	2
3	12	VC application spray filler	07/16/30	10/10/73	43	15	1/2
4	8	PVC production chemical worker	09/04/30	11/25/74	44	17	—
5	11	PVC research dept. lab. assistant	05/09/36	12/16/74	38	5	9
6	7	PVC production shift foreman	01/01/32	01/10/75	43	12	1/2
7	8	Technical appl autoclave cleaner	09/29/26	11/13/75	49	21-1/2	—
8	4	PVC production	01/19/17	12/25/75	58	20-1/3	1-1/2
9	4	PVC production/ processing	08/31/07	10/20/76	—	9-2/3	5-1/3
10	2	PVC production polymerization	12/13/34	Alive	41	14-1/4	Alive
11	2	VC processing	12/29/36	03/77	40	10 until 1971	5-1/2

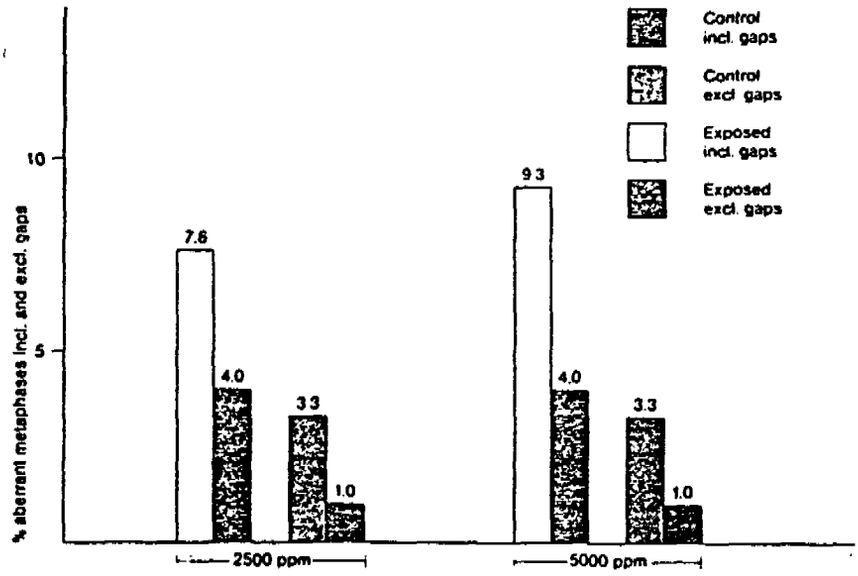

Fig 6. — Chromosome aberration rate of Chinese hamsters after inhalation of 2500 and 5000 ppm VC, respectively.

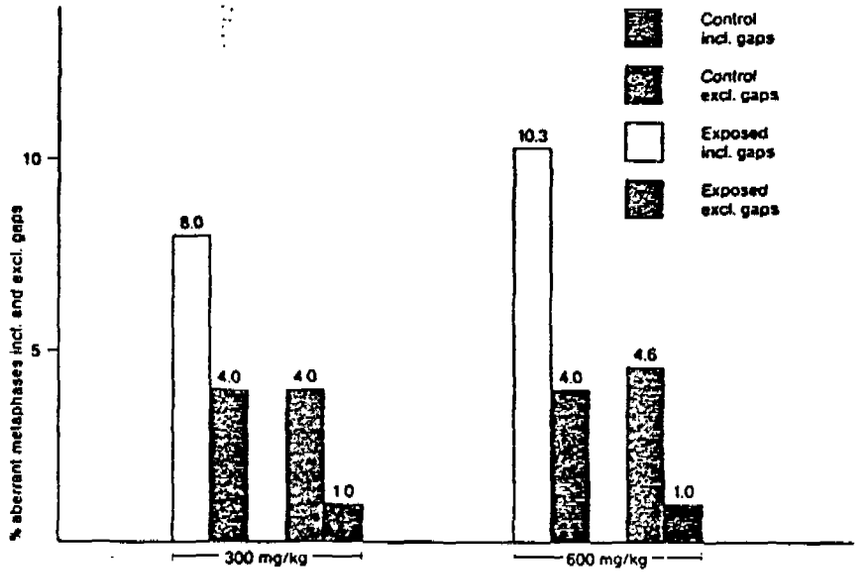

Fig 7. — Chromosome aberration rate of Chinese hamsters after being intraperitoneally injected with 300 mg/kg and 600 mg/kg VC.

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