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Exposure to vinyl chloride monomer: results of a cohort study after a seven year follow up

Agnès Laplanche, Françoise Clavel-Chapelon, Jean-Claude Contassot, Claudine Lanouzière, and the French VCM group (see end)

Abstract

In 1980 a prospective cohort study of exposed and non-exposed subjects was initiated in France by the Institut National de la Santé et de la Recherche Médicale (INSERM U287) in collaboration with occupational physicians from the companies involved. The aim was to evaluate the association between mortality and cancer morbidity and occupational exposure to vinyl chloride monomer (VCM). A total of 1100 subjects exposed to VCM and 1100 non-exposed controls matched for age (to two years), plant, and physician were followed up for seven years (8299 and 8202 person years for exposed subjects and controls respectively) for vital status and health and occupational state. Forty deaths occurred among exposed and 43 among non-exposed subjects (relative risk (RR) = 1.0; 95% confidence interval (95% CI) 0.6-1.5). Forty eight and 32 cases of cancer were reported among exposed and non-exposed subjects, respectively (RR = 1.3; 95% CI 0.8-2.1). Three cases of angiosarcoma of the liver occurred in the exposed group. Eight cases of lung cancer occurred among exposed subjects and six among non-exposed subjects. Fourteen cases of Raynaud's disease were found among exposed and one among non-exposed subjects and the difference was significant. One hundred and twenty three and 93 cases of cardiovascular disease (Raynaud's disease excluded) occurred in the exposed and non-exposed

groups respectively (RR = 1.4; 95% CI 1.0-1.8); this difference was mainly due to hypertension. The test for an increasing risk with increased exposure was significant. The percentages of diseases of the respiratory system did not differ between the two groups (RR = 1.1; 95% CI 0.7-1.8).

In 1980, a prospective cohort study of workers exposed to vinyl chloride monomer (VCM) and non-exposed workers was initiated by the Institut National de la Santé et de la Recherche Médicale (INSERM U 287) in collaboration with occupational physicians from the different companies involved in France. The main purpose of this study was to evaluate the association between mortality and morbidity (especially malignant tumours) and occupational exposure to VCM. This paper presents the health characteristics of the cohort after a seven year follow up.

Materials and methods

The design of the study has been described previously.¹ Briefly, the exposed group consisted of 40 to 55 year old employees exposed to VCM at the time of inclusion in 1980-1 or previously. Controls were employees who had never been exposed to VCM. Each control was matched to one exposed subject for age (to two years), plant, and physician. Interviews for initial data collection were conducted by the physician of each plant over a one year period. Data collected comprised the following information: identification, employment history (the entire work history was coded), occupational category, dates of exposure to VCM, and medical history as well as smoking and drinking habits. The subjects were followed up yearly for seven years from the time of inclusion until December 1988 for vital status and health and occupational state. Causes of death as well as pathological findings were coded according to the 9th revision of the International Classification of Diseases (ICD-9). Subjects who had terminated

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Table 1 Relation between exposure to vinyl chloride and deaths during the seven year follow up

	Deaths during follow up*		RR† (95% CI)
	Yes (n = 87)	No (n = 2115)	
Non-exposed	43	1056	1.0
Exposed after 1976	4	68	1.04 (0.3-3.2)
Exposed < 10 y before 1976	15	405	0.94 (0.5-1.7)
Exposed ≥ 10 y before 1976	21	586	0.99 (0.6-1.7)

*Two subjects were lost to follow up.

†Adjusted for initial characteristics defined in Materials and methods.

Table 2 Causes of death during the seven year follow up

	Exposed (1099)	Controls (1099)
All causes	40	43
Cancer	20	22
Circulatory system	9	10
Suicides	2	4
Traumas	2	0
Alcohol	1	3
Others	1	0
Unknown	5	4

their employment (resigned, been transferred, or retired) were followed up by each physician by mail.

Relative risks (RRs) among exposed compared with non-exposed subjects, are presented for death (all causes), malignant tumour, cardiovascular disease (Raynaud's disease excluded), and pulmonary disease. Subjects with a disease at the beginning of the study were excluded from comparisons concerning that disease. Relative risks were adjusted for age (continuous), duration of activity as a blue collar worker (continuous), foreign origin (yes/no), marital state, (married, single, widowed, divorced), place of residence (urban or rural), paternal or maternal history of cancer, cardiovascular, respiratory, or other disease, smoking habits (non-smoker, current or ex-smoker, number of daily cigarettes, (< or ≥ 20) duration (< or ≥ 25 years)), subject considered ethylic by the plant physician (yes/no), and weekly alcohol consumption (< 14, 14-56, 57-112, > 112 glasses). Relative risks were also expressed according to whether the workers were exposed to VCM for 10 or more years before 1976, exposed for less than 10

years before 1976, or exposed after 1976. The data were verified using the PIGAS system² and analysed with the GLIM package.³

Results

The study population included 1100 exposed and 1100 controls in 12 plants, and has been described elsewhere.^{1,4} The follow up of the two groups was similar. In 1990 the number of person years was 8299 and 8202 and the number of subjects lost to follow up were 2% and 1% in the exposed and non-exposed groups respectively.

MORTALITY

During the seven year follow up, 40 and 43 deaths were reported in the exposed and non-exposed groups, respectively (RR = 1.0; 95% CI 0.6-1.5). Table 1 gives the RRs according to the different levels of exposure. None of the risks differed significantly from unity. Table 2 presents the main causes of death.

MORBIDITY

Exposure to VCM increased the risk of malignant tumour (ICD-9 140-208) slightly but not significantly (RR = 1.3; 95% CI 0.8-2.1). Table 3 shows the relation between the different categories of exposure to VCM and the occurrence of malignant tumour. Table 4 presents the distribution of the 70 sites of malignant tumours. Three cases of angiosarcoma of the liver occurred in the exposed group, but no cases of skin melanoma or malignant brain

Table 3 Relation between exposure to vinyl chloride and occurrence of malignant tumours (ICD-9 140-208) during the seven year follow up

	Malignant tumours*		RR† (95% CI)
	Yes (n = 70)	No (n = 2119)	
Non-exposed	32	1061	1.0
Exposed after 1976	3	69	1.4 (0.4-4.9)
Exposed < 10 y before 1976	14	404	1.2 (0.6-2.4)
Exposed ≥ 10 y before 1976	21	585	1.3 (0.7-2.3)

*Nine subjects had a malignant tumour at entry to the study (three among exposed subjects and six among controls).

†See footnote to table 1.

Table 4 Types and numbers of malignant tumours found during the seven year follow up

	Exposed (1099)	Controls (1099)
All sites (ICD-9 140-208)	38	32*
Lip mouth pharynx (140-149)	1	5
Digestive organs (150-159)	15	12
Oesophagus	1	1
Stomach	2	1
Small intestine	1	0
Colon rectum	5	6
Liver angiosarcoma	3	0
Liver	3	0
Pancreas	0	2
Digestive non-specific	0	2
Larynx (161)	2	2
Lung (162)	8	6
Bone (170)	1	1
Connective tissue (171)	1	0
Melanoma of skin (172)	0	1
Other skin (173)	3	1
Testis penis (186-187)	1	1
Bladder kidney (188-189)	2	3
Endocrine (194)	1	0
Lymphohematopoietic (200-208)	1	2
Secondary (196-198)	1	0
Primary site uncertain (199)	1	2

Subjects without malignant tumours at initial examination (No of subjects at risk): exposed 1096, controls 1093.

*The total exceeds 32 as four subjects exhibited two primary cancers.

tumour. Fourteen lung cancer cases were reported, eight in the exposed and six in the non-exposed group. One case of lymphoma was seen in the exposed and two cases in the non-exposed group.

The risk associated with the onset of diseases of the circulatory system (Raynaud's disease excluded) (ICD-9 390-459) was significantly higher than unity (RR = 1.4; 95% CI 1.0-1.8; $p < 0.05$). Moreover, an increased exposure to VCM was significantly ($p = 0.02$) associated with increased incidence of cardiovascular diseases (table 5). One hundred and twenty three and 93 cases of diseases of the circulatory system were noted in the exposed group and controls respectively. Table 6 presents the types of cardiovascular diseases. Fourteen cases of Raynaud's disease were seen in the exposed and one in the non-exposed group ($p < 0.01$).

The occurrence of diseases of the respiratory system (ICD-9 460-519) did not differ between the two groups (RR = 1.1; 95% CI 0.7-1.8). None of

Table 6 Type of cardiovascular disease (Raynaud's disease excluded) found during the seven year follow up

	Exposed (1099)	Controls (1099)
No of subjects at risk*	933	955
Circulatory system (390-459)	123†	93
Hypertension (401-405)	55	34
Coronary insufficiency (410-414)	30	28
Myocardial infarction (410)	21	16
Cerebral vascular disorders (430-438)	8	10
Arteriosclerosis of limbs (4476, 4479, 4402)	9	11
Other circulatory disorders	27	15

*Subjects without a circulatory disease at the initial examination.

†The total exceeds 123 and 93 as some subjects exhibited two or more circulatory diseases.

the risks significantly differed from unity when different degrees of exposure were considered (table 7).

Discussion

In our study, no relation was found for the occurrence of malignant tumour or respiratory diseases and exposure, whereas an increased risk of cardiovascular diseases was seen in the group exposed to VCM compared with the non-exposed group.

With respect to cancer, the risk of angiosarcoma of the liver has been well documented since 1974. This was confirmed in our study although only three cases were found. During the same period 14 cases of angiosarcoma of the liver were registered in France. This discrepancy can be explained by the limited size of our cohort as well as the selection of the plants and exposed group.

Our results showing a lack of relation between exposure to VCM and other cancers agree with others.³⁻⁴ The IARC study,⁵ which pooled data from Great Britain, Italy, Norway and Sweden totalling 222 746 person years, did not show any positive evidence that a particular malignant disease was a hazard either.

Our findings for the control group are in agreement with French statistics of incidence of cancer⁶ in the same age groups, as 32 cases of malignant tumours were observed, corresponding to an annual incidence rate of 390 per 100 000.

Table 5 Relation between exposure to vinyl chloride and occurrence of cardiovascular disease (except Raynaud's disease) (ICD-9 390-459) during the seven year follow up

	Cardiovascular diseases*		
	Yes (n = 216)	No (n = 1672)	RR† (95% CI)
Non-exposed	93	862	1.0
Exposed after 1976	7	63	0.8 (0.4-1.9)
Exposed < 10 y before 1976	46	327	1.3 (0.9-1.9)
Exposed ≥ 10 y before 1976	70	420	1.5 (1.1-2.1)

*Three hundred and ten subjects had a cardiovascular disease at entry in the study (166 among exposed and 144 among controls).

†See footnote to table 1.

Table 7 Relation between exposure to vinyl chloride and occurrence of pulmonary disease (ICD-9 460-519) during the seven year follow up period

	Pulmonary diseases*		RR† (95% CI)
	Yes (n = 82)	No (n = 1918)	
Non-exposed	37	952	1.0
Exposed after 1976	8	61	1.3 (0.5-3.5)
Exposed < 10 y before 1976	18	381	1.2 (0.6-2.1)
Exposed ≥ 10 y before 1976	19	524	1.0 (0.6-1.9)

*One hundred and ninety eight subjects had a pulmonary disease at entry to the study (88 among exposed and 110 among controls)

†See footnote to table 1.

An increased occurrence of non-malignant respiratory disease as a result of exposure to VCM and polyvinyl chloride dust has been cited. In our study, morbidity from non-malignant respiratory disease was not different among the exposed and non-exposed group. Also the incidence of death from non-malignant respiratory disease was not affected by exposure to VCM in two other recent studies.^{4,7}

By analogy with the effect of other halogenated hydrocarbons, an increased risk of myocardial infarction has been suspected⁸ with, however, little published evidence. One study⁹ showed a slight increase in mortality due to ischaemic heart disease. In our study, although a linear increase in risk of cardiovascular diseases with increased exposure was noticed, no association with myocardial infarction was apparent. The increase in cardiovascular abnormalities is mainly due to hypertension but also to a variety of other circulatory problems including venous disorders, arrhythmia, and valvopathy. This lack of precision makes it difficult to provide an explanation for our positive result. The excess hypertension could be explained by the shift work,¹¹ but this relation has never been found, not even in a study that included 15 000 workers.¹² With respect to Raynaud's disease, 14 cases were seen among the exposed workers (0.2%); this rate is lower than that estimated for the general population—namely, 4%.¹³ The difference can be explained because (1) the disease affects women more than men, (2) a healthy worker effect may exist, and (3) the cases of Raynaud's phenomenon have perhaps been included in the estimation for the general population.

To study the effect of the degree of exposure to VCM, we decided to take into account exposure before and after 1976. This was the year when exposure to VCM was reduced due to the implementation of the 1975 French legislation.¹⁴ Measurements of exposure on workers, or in the workshops themselves, were not available and doses of exposure before 1976 were extremely high compared with doses after 1976.¹⁵ For these reasons, the duration of exposure to VCM was only considered before this date and periods of less or more than 10 years before 1976 were taken as thresholds of low or high exposure.

In conclusion, angiosarcoma of the liver and Raynaud's phenomenon seem to be the major

hazards related to the exposure to VCM. Apart from these diseases, the evidence indicates that the risks associated with exposure to VCM, in terms of both mortality and as morbidity, are probably negligible.

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The persistence of sister-chromatid exchange frequencies in men occupationally exposed to vinyl chloride monomer

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Summary

The persistence of sister-chromatid exchange frequencies in a population occupationally exposed to the well known chemical mutagen vinyl chloride monomer was studied. It was shown that increased values of sister-chromatid exchange frequencies were still present in the lymphocytes of workers who had not been exposed for 8-120 days and retired persons for 5-10 years after exposure. The possible ability of vinyl chloride monomer alkylating metabolites to cause long-lasting damage of the DNA molecule is discussed.

Vinyl chloride monomer (VCM) is a well known carcinogen and mutagen in humans (Anderson et al., 1980; Purchase et al., 1976; Creech and Johnson, 1974; Fučić et al., 1990). After metabolic activation by cytochrome P-450-dependent monooxygenases VCM is transformed into alkylating intermediates which express mutagenic and carcinogenic activity (Bartsch and Montesano, 1975; Zajdela et al., 1980; Barbin and Bartsch, 1989). These intermediates are potent inducers of increased sister-chromatid exchange frequencies (Perry and Evans, 1975; Fučić et al., 1990; Uzych, 1988; Simes et al., 1991).

Sister-chromatid exchange is a selective and

sensitive method for detecting the mutagenic activity of chemicals (Natarajan and Mullenders, 1987) although it has been shown that sister-chromatid exchange frequencies are decreased 4-16 weeks after the end of exposure (Stetka and Wolff, 1976; Uzych, 1988).

The aim of our study was to draw attention to the persistence of changes in the DNA molecule which lead to expression of increased sister-chromatid exchange frequencies after occupational exposure to high concentrations of VCM.

Material and methods

Fifteen plastics industry workers were chosen for the study of sister-chromatid exchange frequencies. As a control population we chose 10 male subjects from the general population 40-50

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years old. In the experimental group the examined workers had been employed in a polyvinyl chloride plant for 1.5–35 years. Those with recent X-ray exposure and drug treatment were excluded from the study. The VCM concentration in the working environment was 2000 ppm. Due to the technological process the concentration could occasionally reach 2000 ppm for a very short period of time.

Sister-chromatid exchange frequencies were measured during and 8, 30 and 120 days after exposure. Retired workers were examined 5–10 years after exposure.

Sister-chromatid exchange frequencies were measured on slides from cultures of blood lymphocytes stimulated with phytohemagglutinin. To 0.5-ml samples of whole blood 8 ml of F-10 medium (Gibco) containing 20% calf serum and 10 μ g/ml bromodeoxyuridine was added. The

cultures were harvested after 72 h. Smoking habits of the individuals were taken into account. For statistical analysis the *t*-test was used (Pavlić, 1970).

Results

Sister-chromatid exchange frequencies in 15 plastics industry workers and 10 control individuals from the general population are shown in Table 1. The periods during which workers had not been exposed were 8, 30 and 120 days. Retired persons had not been exposed for periods of 5–10 years. Two retired persons were examined twice with a 2-year interval. There was no statistically significant difference between sister-chromatid exchange frequencies in workers during exposure and 8, 30 and 120 days after exposure. The range of sister-chromatid exchange frequen-

TABLE 1

COMPARISON OF SISTER-CHROMATID EXCHANGE FREQUENCIES IN SUBJECTS DURING AND AFTER EXPOSURE TO VCM

Subject No.	Employment (years)	Cigarettes/day	SCE frequencies				
			During exposure		After exposure		
			per cell	range	period	per cell	range
<i>Employed</i>							
1	16	0	9.8	6-15	8 days	11.5	7-17
2	12	20	10.7	4-17	8 days	9.1	5-16
3	1.5	0	7.6	4-15	30 days	8.0	4-14
4	12	20	9.7	4-18	30 days	11.1	6-23
5	3	0	8.6	5-14	30 days	8.6	5-15
6	12	0	8.5	6-12	30 days	9.8	4-13
7	2	25	8.5	4-14	30 days	8.5	4-14
8	10	0	9	4-14	30 days	8.3	4-14
9	10	20	9.7	4-19	30 days	9.7	4-18
10	9	20	12.3	5-26	30 days	10.4	4-19
11	15	30	12.7	8-16	30 days	11	5-23
12	20	30	11.1	6-19	120 days	10.9	6-18
<i>Retired</i>							
1	17	15			8 years	10.4	6-20
					10 years	8.6	4-13
2	35	2			5 years	9.5	5-19
					7 years	10.1	5-18
3	7	0			9 years	7.5	5-13
						5.9	0- 7
<i>Control subjects (mean value, n = 10)</i>							

For all calculations 50 cells per person were counted.

cies during exposure was 4-26, and after exposure it was 4-23. Even 10 years after chronic exposure to high concentrations of VCM retired persons showed significantly increased values of sister-chromatid exchange frequencies ranging from 4 to 20. The value of sister-chromatid exchange frequencies for control individuals was 5.9, with a range of 3-7.

Discussion

Sister-chromatid exchange frequencies provide useful information about exposure to chemical mutagens. Although the mechanism of sister-chromatid exchange induction is still a matter for speculation this method is very sensitive and very frequently used in biomonitoring of people occupationally exposed to a wide range of chemicals. It is generally believed that in contrast to severe morphological changes in chromosomes which may persist for a long time, sister-chromatid exchange frequencies in lymphocytes may maintain a high level for 4-16 weeks after exposure (Stetka and Wolff, 1976; Uzych, 1988). The transient nature of increased sister-chromatid exchange frequencies raises the question of the mode of action of the substances that cause them. The question is whether the decrease in sister-chromatid exchange frequencies is due to the disappearance of the chemical agent or its active metabolite from the organism or to the repair process.

Sister-chromatid exchange is a result of a mechanism independent from that which causes chromosome breakage (Wolff and Bodycote, 1975; Lin and Wertelecki, 1982). However, fragile sites are hot spots for DNA recombination as measured by sister-chromatid exchange (Glover and Stein, 1987). This is also confirmed by the observation that chemicals that do not induce gross chromosome aberrations significantly increase sister-chromatid exchange frequencies (Latte, 1974; Perry and Evans, 1975).

Vinyl chloride monomer is a mutagen which increases the frequencies of sister-chromatid exchange in people exposed to high concentrations of it. In contrast to the general opinion today we were able to show increased values of sister-chro-

matid exchange in humans exposed to high concentrations of VCM as long as 10 years after the exposure. Our findings, which demonstrate that there was no significant decrease in sister-chromatid exchange levels from 8 days to 10 years after exposure, present a new aspect of the mechanism of sister-chromatid exchange and the action of VCM. The primary adducts isolated from DNA after exposure to VCM were 3,N⁴-ethenodeoxycytosine and 7-(2-oxoethyl)guanine along with smaller quantities of 1,N⁶-ethenodeoxyadenine (Green and Hathway, 1978; Barbin et al., 1981). The aromatic character of the suspected persistent monoadduct ethenodeoxycytosine ensures its easy formation and stability. The presence of such etheno derivatives in synthetic nucleic acids has been associated with misincorporation of bases during DNA synthesis (Barbin et al., 1981; Hall et al., 1981). The carcinogenic potency of VCM may be related to the persistence of such potentially miscoding lesions. It is suggested that the mode of action of chemicals such as VCM is a multistep mechanism which also includes the formation of interstrand cross-links with either its complementary base or an adjacent base on the complementary strand (Conner and Cheng, 1983). Such cross-links may present yet unknown highly persistent sister-chromatid exchange-inducing lesions. The levels of sister-chromatid exchange even 120 days after exposure to VCM were not decreased regardless of smoking habit. The most interesting subjects were retired persons who, even 10 years after exposure, still carried high levels of sister-chromatid exchanges. The range of their sister-chromatid exchange frequencies did not differ statistically significantly from that in still working subjects.

Consequently it may be assumed that even bone marrow stem cells are affected and that these lesions are not being repaired during the S-phase of the mitotic cycle as described in some hypotheses on the origin of sister-chromatid exchange (Schofield and Lajtha, 1973).

Further investigation is necessary to prove the persistence of alkylated sites in the DNA molecule caused by VCM and detectable by the sister-chromatid exchange method after long exposure to high concentrations of it. At the same time it is

necessary to consider this population as having permanent damage to the genetic pool.

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Filed in Tier I	
PR 0242-4158-100	afatoxins
K-2978	4-aminobiphenyl
	[analgesics containing]
	phenacetin
	azathioprine
No #	[197] benzene
K-2661	benzidine
K-10149	betel quid and tobacco
No #	bis(chloromethyl)ether
K-1729	chlorambucil
No #	chlornaphazine
No #	cyclophosphamide
OK-001-5505	cyclosporin *
No #	ethanol *
K-2544	melphalan
No #	MOPP
No #	mustard gas
K-73932	myleran
K-46770	2-naphthylamine
K-2089	thiotepa *
K-47442	tobacco, [smokeless]
DD-168	tobacco smoke
No #	treosulphan
No #	vinyl chloride
K-1711	
Tars and oils	
TX-665	coal-tar pitches
K-19199	shale oil
Hormones	
K-4998	diethylstilbestrol
Metals	
K-3154	[arsenic] compounds
K-9154	[chromium] compounds
DD-34	[nickel and nickel compounds]
Fibers	
K-2826	[1912] asbestos
No #	talc containing asbestiform fibers
Industrial process	
No #	aluminum production *
Other	
No #	ionizing radiation *

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