Vinyl chloride monomer and hepatocarcinoma

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Introduction

Chronic exposition to vinyl chloride monomer (VCM) is recognized as cancerogenous for human beings by the International Center of Research against Cancer. VCM may be responsible for the development of hepatic angiosarcomas (HAS). This property was not recognized before 1974. The mechanisms for its hepatotoxicity are nowadays well established (1).

May professionnal contacts with VCM induce a hepatocarcinoma (HCC) ?

The answer to this question is much more difficult for HCC than for HAS. Indeed, angiosarcoma appears rarely spontaneously. Its yearly incidence, in the absence of contact with any known carcinogen, is evaluated to 0.0014 cases for 100.000 inhabitants in the United States by the National Cancer Institute (2) ; a necropsic series found only 1 case for 50.000 autopsies within 44 years. Its frequency is increased by 400 fold in case of important and prolonged exposition to VCM (3).

At the contrary, HCC is a frequent tumor ; in the majority of the cases, the underlying disease is cirrhosis. Its incidence in the United States and in Europe is comparable and estimated at 3 cases a year for 100.000 people ; in countries where hepatitis B virus is endemic, this annual incidence rises to 30/100.000, or even more (4,5). Chronic infection by hepatitis C virus represents also a major risk for the development of HCC ; in Italy, Spain and Japan, 50 to 75% of the hepatocarcinomas are related to a chronic infection by this virus (6). One to 3% of the people in the industrialised countries is carrying hepatitis C virus (7) ; 20% of them will develop cirrhosis after a 20 years evolution. The yearly risk for the development of a hepatocarcinoma in a cirrhotic patient, whatever the cause of the cirrhosis, fluctuates between 1 to 4%.

Before linking repeated exposition to a professionnal toxic substance and the occurrence of a HCC or of any other type of cancer, it is important to search for other clearly established risk factors.

In order to answer clearly to the above question, it is valuable to remind how the relation between VCM and angiosarcoma was demonstrated and to see if, by applying the same methodology (animal experiments compared to human epidemiology), a similar relationship between VCM and HCC may be suggested.

Processing and utilization of vinyl chloride monomer

Vinyl chloride (or monochloroethylene : CH2 = CH2Cl) is an aliphatic hydrocarbure ; it is a colorless inflammable gas under ambient conditions. Its low molecular size allows an easy absorption by biological membranes (8) ; after inhalation, its diffusion is quick. Industry uses vinyl chloride polymers, called resins, to produce plastics. The first manufactures that processed these polymers were built in the United States and Germany in the thirties.

VCM is synthetised by hydrochlorination of acetylene (this process was almost exclusively in operation up to 1960) or by oxychlorination of ethylene to 1,2-dichloroethane which is then cracked to vinyl chloride and HCl by subsequent pyrolysis. Gaseous VCM is stored in large containers and piped into large polymerisation reactor vats through a closed system. Its polymerisation begins in these containers. At the end of this process, unreacted gaseous vinyl chloride monomers are recovered and recycled ; polymerised material is dropped into secondary tanks and used in the plastic processing.

At the beginning of the production of VCM, the risk of contact with this gaseous monomer was possible during the polymerisation phase (gastight canalizations) or, the most often, after polymerisation, when reactors were opened and cleaned. Until the late 1960s, this cleaning process was done manually by a man lowered into the reactor for that purpose (9). VCM air concentration could reach 10.000 ppm when the reactor was opened. Polymer residues, sticked to the walls, when taken off by the autoclave cleaner, released VCM bubbles, exposing the worker to 1.000 ppm toxic gas (3). Since 1970, high-pressure water hoses are used for cleaning reactors. From 1975 until now, the level of VCM tolerated in the surroundings of the polymerisation machines is maintened very low and must be less than 3 ppm on annual average (European Communities recommandations).

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Table 1 shows the decrease of VCM exposure for workers in polymerisation plants with time (10,11).

Table 1

1945 – 1955 : 1000 to 2000 ppm
1955 – 1959 : 500 to 1000 ppm
1960 – 1970 : 300 to 400 ppm
1970 – 1973 : 150 ppm
➢ 1974 : < 5 ppm

Biotransformation of VCM

VCM metabolism occurs in the liver. It is absorbed by the hepatocyte and transformed into chloracetaldehyde (CAA) by an alcohol deshydrogenase and to chloro-ethylene (CEO) by P450 E1 monooxygenases (12). CEO and CAA are susceptible to bind to DNA nucleotides, forming mutagenic addicts (responsible for nucleotides substitutions). CEO is more genotoxic than CAA (13). CEO is detoxified by conjugation with sulhydril groups, using a glutathione transferase. CEO metabolites are excreted by the kidney (14). Genetic variations occur in the activity of the P450 E1 (more active, it leads to more CEO) and in the family genes coding for the glutathione transferases (less glutathione leads to more CEO). The effect of MCV exposure may thus also depend from individual genetic variability (15).

A chromosomic analysis on animals in contact with MCV revealed alterations ; however, it was impossible to establish a relation between these chromosomic alterations and tumor occurrence (16).

VCM-induced gene modifications into the liver may be potentiated by alcohol consumption, that takes alcohol deshydrogenases for its proper use and favours in that way the P450 route for MCV degradation, leading to more CEO. Moreover, alcohol decreases glutathione activity (8). In rats, ethanol ingestion combined to VCM inhalation have a synergistic effect in inducing liver angiosarcoma (17). Metabolic products from VCM, as CEO, may travel from a hepatocyte to an adjacent sinusoïdal cell, leading to a malignant transformation of that cell (18).

Finally, into the liver, other cell types than hepatocytes have the possibility to degradate VCM, but in a lesser effective way (19).

Animal toxicity of VCM

The first animal experimentations, from 1930 to 1963, concerned only the acute possible toxicity of VCM. Cardiac rythm disturbances, comas, even deaths were reported (20). No liver disease was described.

Chronic exposure to VCM, mostly in rats, revealed hepatic toxicity. Animals presented with hepatomegaly ; pathologic examination disclosed hepatocyte swelling, steatosis, centro-lobular necrosis and fibrosis. The first papers on that purpose were published in 1961 (20,21).

The first experimentation revealing a potential carcinogenic effect of VCM used doses as high as 30.000 ppm a day for 1 year (22) ; the rats developed skin tumors, mostly in the paraauricular region (Zymbal gland tumors), bronchial carcinomas and osteochondromas. No liver cancer was found. Because of these preliminary observations, the oncogenic power of the substance was carefully studied, from 1971 to 1975, in laboratory animals, receiving inhaled, ingested or injected MCV at daily doses from 50 to 10.000 ppm (7.000 rats, hamsters and mice) (23). Induced tumor types differed from one species to another. However, hepatic angiosarcomas were observed in all 3, when they were receiving more than 50 ppm of MCV a day (table 2). A dose-response relationship (dose = concentration x exposure time) was obvious for angiosarcomas. The most important parameter was the length of exposure to MCV. Hepatocarcinomas were also found in rats, less frequently than angiosarcomas, for doses from 500 to 10.000 ppm. Lower doses (50 to 250 ppm) were able to provoke angiosarcomas, but not hepatocarcinomas (table 2).

Table 2. — **Rats : exposition to VCM by inhalation 4 hours a day, 5 days a week, for 52 weeks ; occurrence of hepatic tumors (from Maltoni, 23)**

VCM exposure	Number of rats	HAS	Latency HAS (in weeks)	HCC
10.000 ppm	61	9	64	1
6.000 ppm	60	13	70	1
2.500 ppm	59	13	78	2
500 ppm	59	7	81	4
250 ppm	59	4	79	0
50 ppm	59	1	135	0

The new-born rat developed more easily a HCC than an older animal (24,25).

In another study (26), angiosarcomas and "cholangiomas" are reported in rats inhalating also 500 to 20.000 ppm a day for 1 year ; the "cholangiomas" were only observed with doses of 20.000 ppm. These tumors seem to be either hepatocellular carcinomas or cholangiocellular carcinomas. Once again, no tumor was found in animals receiving up to 50 ppm .

Following the above experiments, a rodent exposed daily for 1 year at doses above 50 ppm may develop a neoplastic tumor of the liver, the most often an angiosarcoma (13% of the cases) but also a hepatocellular or a cholangiocellular carcinoma (23,26).

Human toxicity of VCM

The effects of an acute VCM intoxication have been recognized a long time ago in man as well as in

animals and include drowsiness and cardiac arrhythmias that may lead to death (27,28).

The first chronic liver disease attributed to VCM was reported as soon as 1949, among workers in a polymerisation plant in USSR : 73 presented with painful hepatomegaly without jaundice. The outcome of these men is unknown. The paper was written in Russian and ignored abroad (29).

In 1963, Romanian physicians published their observations about 168 VCM workers followed for 4 years (30). The intensity of exposure was not mentionned. Raynaud phenomenons, dermatitis, sclerodermia, ... and 1 hepatomegaly were reported in 51 men. Two had a liver biopsy, revealing fibrosis.

In 1965, another Russian paper reported a hepatitis attributed to VCM (31).

Acro-osteolysis of the distal phalanges was described for the first time in Belgium in 1966 (32), and quickly thereafter in Great-Britain (33) and in the United States (34). The way of processing VCM was then modified, to reduce MCV exposure. This measure decreased the number of acro-osteolysis.

At the end of the sixties, the "VCM disease" included acro-osteolysis, Raynaud phenomenon and skin lesions. A possible liver disease remained ignored.

The relationship between VCM exposure and HAS was for the first time suggested in man in December 73 : the autopsy of a VCM worker revealed a liver angiosarcoma responsible for death. This case was the third one in 3 years among the employees of that factory (35). The human cases were published before the animal experiments demonstrating the hepatocarcinogenetic power of VCM (23).

The causes of death of VCM workers were retrospectively reviewed (2,9,36). No hepatic tumor other than angiosarcoma was reported until 1976. Careful examination of VCM workers confirmed the existence of a hepatomegaly, often associated with a splenomegaly, in 10% of them (20,37).

Until December 1997, 196 angiosarcomas of the liver, in relation with prolonged exposure to VCM, were reported. Not any new case was found in West Europe and in the US since the decreased risk in exposure (since 1974).

In all the patients in whom HAS developed, exposure time to VCM was long : from 10 to 33 years (mean 18 years) (38). Latency periods (from the first exposure to the diagnosis of the tumor) were between 9 and 35 years (mean 25 years).

Sixteen cases of hepatocarcinomas have also been associated with VCM exposure ; as for HAS, they have been detected in workers heavily exposed before 1974. The mean period of exposition was 18 years (from 4 to 43 years). The mean latency period was of 25 years. The first case was published in 1976 (39), the last in 1997 (40). VCM was also made responsible for the occurrence of a cholangiocellular carcinoma (41). The role of VCM in the appearance of these carcinomas is detailed in the discussion.

Hepatic histological lesions associated with VCM

Hepatic cells include hepatocytes, biliary cells, sinusoidal cells, Kupffer cells and stellate cells able to differentiate into fibroblasts (42).

VCM may induce 3 different types of hepatic tumors : the hepatocellular carcinoma (arising from hepatocytes), the cholangiocellular carcinoma (arising from the cells lining the biliary canaliculi) and the angiosarcoma (arising from the vascular sinusoidal cells). Hepatocytes and biliary cells derive from the same stem cell ; mixed tumors (hepato- and cholangiocellular carcinomas) have been described (43).

Before ascertaining that an environmental factor is responsible for a disease, it is imperative to identify precisely the types of lesion it may provoke, to link this factor to the observed abnormalities, to search for a "precursor lesion", to understand the pathogenic mechanism and to evaluate the incidence of the disorder.

As soon as 1975, the liver deleterious effects of VCM were suggested by Popper (37), who reviewed retrospectively liver biopsies and necropsic material from 11 workers of polymerisation plants. The succession of the histological lesions helps to the understanding of the neoplastic process.

On electronic microscopy, at the initial phase of exposure to VCM, before the appearance of abnormalities on optic microscopy, hypertrophy of the smooth endoplasmic reticulum and a loss of villosities in the hepatocytes are the first manifestations of VCM toxicity on cellular membranes (8).

On conventional microscopy, the first modification attributed to VCM is also found in the hepatocytes : diffusely throughout the parenchyma, some hepatocytes are remarkable with their enlarged size, and the hypertrophy of their nucleus. Theses pictures of *focal hepatocyte hyperplasia* are reversible if VCM exposure is interrupted (44).Later on, endothelial cells lining the sinusoids swell and prolifere, as the hepatocytes did before ; this endothelial proliferation is more prominent close to areas of activated hepatocytes. In the same time, in the space of Disse, Ito cells initiate their differentiation into fibroblasts. The observation of this mixed focal hyperplasia (abnormal hepatocytes and sinusoidal cells) means a evolution to irreversible liver damage (45).

VCM is also reponsible for an early subcapsular, portal and perisinusoidal fibrosis. Usually, the lobular architecture is preserved ; however, evolution to cirrhosis has been described (37,46). The development of liver fibrosis is leading to splenomegaly and sometimes to portal hypertension.

If the exposure to VCM is going on, sinusoidal dilatation becomes prominent ; sinusoidal cells undergo cytoplasmic and nuclear modifications. This dedifferentiation is the first step to a malignant transformation leading to angiosarcoma. Indeed, von Willebrand factor (47) and specific vascular membrane antigens (48) may be detected into these liver angiosarcomas.

The activated hepatocytes may also evolve to neoplastic cells.

The VCM-induced lesions follow the same succession scheme in man and the animal (44,49) ; they are specific and reproducible (8,50).

Chemical products other than VCM may modify the liver architecture in the same way.

Chronic intoxication with arsenic and with thorium dioxyde (Thorotrast®) induces focal activation of hepatocytes and sinusoidal cells, as well as fibrosis, like VCM does (51). Arsenic is one of the components of the Fowler solution, used previouly to treat psoriasis. Some patients treated with this solution died because of an angiosarcoma ((52). Arsenic-containing pesticides used by wineyards were known to be able to induce angiosarcomas and, less frequently, hepatocarcinomas (53,54).

Thorotrast®, used as contrast medium in radiology, was taken for responsible of hepatic fibrosis, angiosarcomas and hepatocarcinomas in man (55,56) and animals(57).

The prolonged use of androgenic steroids is also associated to the occurrence of liver angiosarcomas ; in the surroundings of the tumor, focal mixed hyperplasia and sinusoidal dilatation heve been described (58). Moreover, these steroids, known for favouring the development of hepatic adenomas, may also lead to hepatocarcinomas (59) and sometimes to cholangiocellular carcinomas (60).

The combination of hepatocytic hyperplasia and activation of sinusoidal endothelial cells, together with sinusoidal dilatation, is thus characteristic of a hepatic toxicity of chemical agents such as vinyl chloride monomer, arsenic, thorium dioxyde or androgenic steroids ; it may be responsible for a malignant transformation of both cell lines, leading to the occurrence of angiosarcomas or hepatocarcinomas.

Symptomatology and biological disturbances in relation with VCM-induced hepatic disease

Acute intoxication with VCM provokes easily recognisable symptoms. A short accidental contact with high doses (8.000 to 10.000 ppm) gives cutaneous lesions (the most often burnings), digestive disturbances as nausea and abdominal pain, reversible acute liver necrosis, neurological symptoms (sleepness, even coma) and cardiac arrhythmia. Repeated expositions to lower doses (150 to 250 ppm), for at least 10 years, may cause fatigue and drowsiness. The liver may be enlarged and painful at palpation. Liver tests (alkaline phosphatase, ALT) are normal, or slightly elevated (61). Hepatic biopsy shows focal hepatic hyperplasia, which is reversible. If the liver tests are abnormal, they go back to normal within some months if there is no contact anymore with VCM. They will be elevated again if the exposure to VCM is repeated (62).

An exposure of more than 10 years, with doses above 250 ppm, is responsible for chronic hepatic lesions, irreversible if the liver biopsy discloses mixed focal hyperplasia (of the endothelial cells and of the hepatocytes). This chronic liver disease remains silent for a long period. Hepatomegaly and splenomegaly become often obvious at physical examination.

The first clinical symptoms are in relation with portal hypertension, observed in 20% of the patients suffering from VCM-induced chronic liver disease ; the most often reported symptom is digestive bleeding from esophageal or gastric varices (20,62,63).

It is frequently the enlarging liver tumor that causes the first symptoms : weight loss, painful enlarged liver, sudden abdominal pain due to intraperitoneal tumor rupture, ... The tumor may remain asymptomatic and be then detected on routine examination (64).

According to Tamburro (8), a VCM worker with a chronic VCM-induced liver disease, may develop a liver cancer 20 years after the first exposure to the substance.

Laboratory tests are of little help in the diagnosis of VCM-induced liver disease. Transaminases and alkaline phosphatase, as bilirubin, remain often normal. The dosage of biliary acids (65) and indocyanin green clearance (66) are early markers of hepatic dysfunction. Alpha fetoprotein remains normal if an angiosarcoma develops and is not systematically elevated when a hepatocarcinoma occurs.

Other tumoral markers such as proteins p53 and p21 may be helpful. Indeed, 2 steps are often necessary for neoplastic transformation : a mutation on chromosome 12 allowing the activation of the oncogene Ki-ras, and a modification on chromosome 17 that reduces the function of a suppressor gene coding for p53. Oncogenes activation is increased when cells (hepatocytes or sinusoïdal cells) are replicating, as it is the case during chronic exposure to VCM. This replication is also enhanced when liver cells are damaged, as in cirrhosis (67).

Binding of CEO (a metabolite of VCM) to hepatic cells may be responsible for a mutation on the codon 13 of the Ki-ras gene, reported in the majority of the VCM-induced angiosarcomas (68,69). This activated gene has also been recognized in colo-rectal cancers, pancreatic and bronchial adenocarcinomas ; the modified codon was the twelfth in these cases. This mutation of the Ki-ras gene induces the synthesis of an abnormal protein, called p21, detectable in the tumor (70) and in the serum of people developing VCM-induced angiocarcinomas. The p21 protein may be found before the development of angiosarcoma ; its presence is correlated with the

length of VCM exposure (71). This p21 protein was not detectable in the unique analysed case of VCM-attributed hepatocarcinoma.

The p53 protein acts as a tumor suppressor. In a normal situation, it is bound to specific DNA sequences, to decrease cellular growth. This function is important to maintain genomic integrity. A modified p53 loses its capacity to bind DNA and thus also its suppressor role. The suppressor gene coding for p53 is mutated in 50% of the cancers ; some codons are more often modified, as the codon 249 in the aflatoxin-induced hepatocarcinoma. The mutated p53 is antigenic ; antibodies anti-p53 may be detectable in tumors and in serum (72). These antibodies exist in 50% of the angiosarcomas (73) and in 33% of the corresponding sera (69). They may be detected some months before angiosarcoma is evident (74). This p53 mutation was present in 1 out of 8 studied VCM-related HCC (75). Anti-p53 have been found in the serum of patients with hepatocarcinoma unrelated to MCV (76).

Anti-p21 and/or anti-p53 in the serum might thus be early markers of evolution to angiosarcoma. Hepatocarcinoma may also manifest by the secretion of a mutated p53 protein ; only a small number of patients with HCC have been evaluated until now.

Discussion

Chemical carcinogens, as arsenic, Thorium dioxyde, anabolic steroids and vinyl chloride monomers are responsible for liver damage ; they induce the same kind of lesions. Foci of hepatocyte hyperplasia appear first, followed by endothelial cell proliferation in the sinusoids. Fibrosis occurs, without disturbance of the lobular architecture, at least at the beginning and may induce portal hypertension. The hepatic lesions lead to tumor growth after a long period of time (> 10 years).

The most often reported tumors derive from endothelial cells : usually angiosarcomas, rarely hemangioepitheliomas.

Arsenic, Thorium dioxyde and steroids may also initiate neoplastic transformation of the hepatocyte, evolving to hepatocarcinoma. This filiation has been demonstrated in the animal as well as in humans. It is probable that VCM can also induce hepatocarcinoma, because it leads to the same sequence of events than the 3 chemical substances mentionned above. Indeed, experimentally, VCM may be responsible for the occurrence of HCC in rats, mostly if an important exposition (6.000 to 10.000 ppm) occurs in the perinatal period (24).

To establish a link between the chemical agent and the existence of a tumor, it is worthwhile to observe, in the liver distant to the tumor, the disturbances that characterize a chronic VCM-induced liver disease.

The relation between VCM and a hepatocarcinoma is easier to establish in the absence of other factors associated with the occurrence of this tumor. The most common cause of HCC is cirrhosis. In 90% of the cases, the extratumoral liver is cirrhotic ; cirrhosis is indeed considered as a preneoplastic state. The carcinoma is considered as the consequence of the malignant transformation of regenerative nodules. Yearly incidence of HCC in a cirrhotic liver is estimated from 1 to 4%. Cirrhosis, whatever its origin, is a frequent disease : its prevalence is 2.000 cases for 1 million people. The most often, the cirrhosis is due to a chronic viral infection (viruses B or C) or to an excessive prolonged alcohol consumption. HCC occurs in 15 to 20% of patients suffering from hepatitis B-related cirrhosis, 20 to 40 years after contamination.

The prevalence of hepatitis B in European western countries and in the United States is less than 7%.

Chronic hepatitis C infection is more frequent in these regions : 1 person out of 100 carries chronically this virus. Chronic hepatitis C leads to cirrhosis in 20% of the infected individuals after 20 years of evolution. Once cirrhosis constituted, the risk of hepatocarcinoma development is 1 to 4% a year, as for hepatitis B chronic infection (6). Alcoholic cirrhosis may also be complicated by the development of HCC, even if this cirrhosis is asymptomatic and alcohol consumption stopped.

VCM induces the most often fibrosis without cirrhosis. The occurrence of HCC in a VCM worker, whom liver histology reveals, in the absence of cirrhosis, lesions characteristic of VCM-related disease (hepatocytic or mixed hyperplasia, sinusoïdal dilatation, subcapsular and sinusoïdal fibrosis) is probably related to VCM. Absence of cirrhosis, even if the pathognomonic lesions of VCM disease are lacking, is also in favor of the causative role of VCM. Without cirrhosis, markers for hepatitis B or C viral infection will be missing, as heavy alcohol consumption.

Conversely, the occurrence of HCC in a patient with a cirrhosis of viral or alcoholic origin should be related rather to the virus or to alcohol consumption, even if he has been exposed to VCM (77). VCM may act as a cofactor, inducing more easily the neoplastic transformation, by producing adducts with the hepatocytic DNA. However, the synergistic effect between VCM and alcohol has been demonstrated only for the induction of angiosarcomas, and solely in animals (17) ; such link has not been reported for HCC, neither in animals nor in men.

Analysis of published cases of HCC, probably related to VCM

Sixteen human cases of hepatocarcinoma must be considered as due to VCM (table 3).

All patients were exposed to VCM for 8 to 43 years (mean : 16 years), before 1974, at doses above 50 ppm, and often very elevated, as all these patients worked in polymerisation plants within the years 50 and 60. Indeed, at that time only a few precautions were taken to

Table 3. — **Published cases of hepatocarcinomas probably VCM-related**

Year	author	age	exposure (years) (years)	delay of exposure	cirrhosis	VCM disease	HAS	HBV markers	HCV markers	alcohol	alpha FP
1976	Gokel	67	23	26	no	yes	no	ND	ND	ND	ND
1978	Delorme	ND	ND	ND	ND	ND	yes	ND	ND	ND	ND
1979	Pialat	53	15	ND	no	no	no	ND	ND	no	ND
1981	Koischwitz	54	19	20	yes	no	yes	ND	ND	no	ND
1982	Jones	60	ND	ND	no	yes	yes	ND	ND	ND	ND
1983	Evans	54	8	17	no	yes	no	ND	ND	ND	ND
		48	12	18	no	yes	yes	ND	ND	ND	normal
1985	Dietz	67	20	23	no	ND	no	absent	ND	no	ND
		54	26	?	no	yes	no	absent	ND	no	normal
		55	13	15	no	ND	no	absent	ND	no	normal
1996	Lelbach	54	19	20	yes	yes	yes	absent	ND	no	normal
		61	43	44	no	ND	no	absent	ND	ND	normal
		66	19	34	no	yes	no	ND	ND	ND	normal
		48	10	17	no	yes	no	ND	ND	ND	normal
1997	Saurin	51	11	31	no	yes	no	absent	absent	no	normal
		60	13	31	no	yes	no	absent	absent	no	elevated

(ND : data not available ; HAS : presence of an angiosarcoma, HBV : hepatitis B virus, HCV : hepatitis C virus).

reduce the exposition. Seven workers out of the 12 whose precise job was reported were autoclave cleaners. The latency period (the interval between the first exposition and the diagnosis of the tumor) ranged from 15 to 44 years, with a mean of 25 years ; this latency period was longer than the one generally observed for angiosarcomas (40). The age when the precise diagnosis was done was comprised between 48 and 67 years (mean : 57 years). Only men developed the disease : indeed, women were not employed for VCM polymerisation.

1) The first case was reported in Germany (39). The patient was 67 years at the time of the diagnosis, made at autopsy. Working at the polymerisation from 1949 to 1972, he was submitted to more than 50 ppm until he retired. Histological examination of the liver revealed the absence of cirrhosis but septal and subcapsular fibrosis ; hepatocytic hyperplasia was also observed. These lesions are typically seen in VCM-associated liver disease. HbsAg was absent ; hepatitis C virus was still unknown.
2) The second case, published in 1978, originated from Quebec : the man died in 1971 from hepatic insufficiency, after having been exposed to VCM for 23 years, at unprecised doses (78). The tumor infiltrating the non-cirrhotic liver had elements both from hepatocellular carcinoma and angiosarcoma. Portal and perisinusoidal fibrosis, sinusoidal dilatation and endothelial cells dysplasia suggested that the disease was induced by VCM. This case is the only one reported in whom the tumor had the characteristcs of both a hepatoma and an angiosarcoma.
3) The third case, reported in 1980, was the one of a Frenchman who worked for 15 years as autoclave cleaner before 1970 (3). He developed a well differentiated hepatocarcinoma on a non-cirrhotic liver. Biopsies outside the tumor were not realized. Markers for hepatitis B virus were not asked for. Even so, the absence of cirrhosis strongly suggests a filiation between VCM and the occurrence of the tumor.
4) A German case published in 1981 (79) is of difficult interpretation, because of the presence of a cirrhosis and the absence of sinusoidal distorsion. This worker cleaned the autoclaves from 1955 to 1965, and afterwards worked for 10 more years at polymerisation. He died 4 years later, from 2 distinct tumors (1 hepatoma and 1 angiosarcoma). Histology of the non-tumoral liver disclosed only septal and subcapsular fibrosis. Did the cirrhosis provoke the hepatocellular carcinoma, and VCM the angiosarcoma, or did VCM induce the cirrhosis (rarely reported) and consecutively the 2 tumors ? The absence of data concerning possible viral infection or alcohol consumption hampers any definitive conclusion.
5) In 1982, in the United Kingdom, the autopsy of an autoclave cleaner, doing that job for 7 years before 1969, and died from esophageal variceal hemorrhage, allowed the discovery of 3 different kinds of tumors developed in a normal liver : several foci of angiosarcoma, an adenoma and a hepatocellular carcinoma (45). Fibrosis and "atypic" sinusoidal cells were mentioned without further details. Vinyl chloride monomer pathogeny in the development of these tumors was probable : the simultaneous formation of distinct cancers from hepatocytes and sinusoidal lining cells seems conceivable.
6) Two polymerisation workers, exposed to VCM 8 and 12 years before 1974, deceased in 1983 from hepatic failure and were autopsied (80). At 48 years, one had a hepatocarcinoma and an angiosarcoma and the other, at 54 years, a hepatocarcinoma. They were not cirrhotic. The repartition of fibrosis and the presence

of foci with hepatocyte hyperplasia suggested that VCM induced the liver disease. Blood alpha fetoprotein dosage — reported for the first time — was normal.

7) Three new HCC were reported in 1985 (81) in workers exposed to VCM from 13 to 26 years, between 1952 and 1982. These tumors were observed in non-cirrhotic livers, from patients who did not drink alcohol in excess and without markers of hepatitis B virus infection. Unfortunately, no precise histological description of the liver parenchyma surrounding these tumors was given. Alpha-foeto protein levels were normal.
8) In 1996, Lelbach (41) put together previous cases of workers heavily exposed to VCM. Four developed a hepatoma after exposition from 4 to 19 years to high doses of VCM ; three were autoclave cleaners. The first patient died from cirrhosis maybe due to iron deposition ; he was not alcoholic and had no HBV markers. Autopsy revealed an angiosarcoma and a distinct hepatoma, in a cirrhotic liver showing the characteristic lesions induced by VCM esposition. Again, it was impossible to precise if the hepatocarcinoma resulted from the exposure to a chemical carcinogen or was related to the cirrhosis, possibly provoked by a disturbance in iron metabolism. Two other hepatocarcinomas occurred on normal livers ; one patient exposed from 1942 to 1977 had only septal fibrosis at autopsy, the other, who worked in the polymerisation plant from 1954 to 1973, suffered from portal hypertension without cirrhosis at biopsy. The last patient worked as an autoclave cleaner, for 4 years (up to 1972) ; he stopped this work because of acro-osteolysis and a Raynaud phenomenon. A liver biopsy performed at that time because of hepatomegaly and BSP retention, revealed perisinusoidal and portal fibrosis associated with endothelial cell proliferation. Two years later, skin lesions were healed but portal hypertension developed, as esophageal varices were found. In 1987, when the man was no more exposed to VCM for 15 years, routine liver ultrasound revealed a mass in the right lobe. Laparotomy disclosed the absence of cirrhosis and a biopsy was in favor of a cholangiocellular carcinoma, responsible for death in a few months. Alpha foetoprotein levels remain normal in the 4 cases.
9) Finally, in 1997, 2 more hepatocarcinomas were published in VCM workers. Their diagnosis was made in 1990. In the parenchyma around the tumors, the typical lesions associated with VCM were observed. These patients had no markers for HBV or HCV infection. Alpha foetoprotein was above normal values in one. Both patients were surgically treated and alive when the paper was published : they are the only ones who survived between the 16 reported carcinomas. In these 2 last cases, the relationship between VCM and the occurrence of hepatoma is obvious.

Analysis of the published cases of hepatocarcinomas where the relation between VCM and hepatocarcinoma occurrence is doubtful

In 1975, Popper mentioned the case of a worker exposed to VCM for 12 years and who developed a hepatocarcinoma. The possibility of another precipitating cause was not evoked. The same year, 2 other cases were reported ; when the histology of these tumors was carefully reviewed, it became clear that those masses were angiosarcomas.

The death certificates among 7000 English VCM workers were retrospectively reviewed (83) ; four of them mentioned the existence of a hepatic tumor : 2 were angiosarcomas, and 2 others "non angiosarcomas" (83). No argument is valuable to make a link between these lesions and VCM, as nothing is known about the nature of these lesions — they may be metastases — and about an eventual underlying disease.

A paper published in France in 1977 made a correlation between the occurrence of a hepatocarcinoma on the cirrhotic liver of a VCM worker and the exposure to VCM for 14 years, without giving any detail concerning the histology of the liver and the medical history of the man ; the data are too scarce to establish any relation between VCM and the hepatic tumor.

Another retrospective study from 1990 (85) among Italian VCM employees found that hepatic tumors were the cause of death in 14 of them. Seven suffered from a angiosarcoma, 2 had a hepatocarcinoma and 5 had a tumor whose histology was not available. Mean exposition duration to VCM was 20 years, but the period was not precised. No detail was given concerning alcohol consumption or chronic viral liver disease. The establishment of a link between these 2 histologically proven hepatocarcinoma and VCM remains uncertain.

The results of an epidemiologic European survey, undertaken to determine a dose-response relation between the occurrence of a liver cancer and VCM exposure, were published in 1991 (86). The frequence of a liver cancer was 3 times higher in VCM workers than in a matched control population. Seventeen autopsic reports were evaluable : they revealed an angiosarcoma in 16 cases, and 1 hepatocarcinoma ; no detail was given about this last case, especially concerning other factors that may induce the occurrence of a hepatocarcinoma.

Finally, a paper published in 1998 reviewed the outcome of 2224 VCM workers in Taïwan (87). Twelve hepatic tumors were diagnosed ; six were hepatocarcinomas (4 with a histologically proven diagnosis, 2 with a high level of alpha foetoprotein) developing in patients who were chronic carriers of hepatitis B virus. The remaining 6 non characterised hepatic masses grew in 4 HBV-carriers, in 1 alcoholic cirrhosis and in 1 patient having a cirrhosis of unknown origin. Identification of possible HCV carriers was not made. The histology of the non-tumoral liver was never reported. It is thus

impossible to precise if the hepatoma was due to a chronic B or C viral infection, to heavy alcohol consumption or to a VCM-related liver disease. The age of tumor occurrence was 51 years, corresponding to a latency period of 20 years (between the first exposure to VCM and the diagnosis of a tumor) but also to the mean age of appearance of a hepatocarcinoma in a region of the world where hepatitis B virus is endemic (20% of the population in Taïwan, which was the prevalence of this disease found among the workers included in the study).

If, as proposed by the authors of this last paper, VCM was reponsible for the occurrence of the reported hepatocarcinomas, it is strange that no angiosarcoma was diagnosed, as this tumor occurs ten times more frequently than hepatocarcinomas in VCM workers. It is probable that the hepatic tumors reported in this study were more in relation with chronic HBV carriage than with VCM exposure.

A synergistic effect between HBV and VCM may exist : indeed, the authors pointed out that VCM workers infected by HBV suffered more frequently from hepatocarcinoma than HBV infected workers without professional contact with VCM.

Toxicological, clinical and epidemiological data discussed here above suggest that a hepatocarcinoma may be induced by a professionnal exposure to VCM ; however, all the hepatocarcinomas described in VCM workers may not necessarily be attributable to it. The data concerning the duration of exposure to VCM are scarce. Anyway, we know that hepatocarcinomas may appear after an exposure of at least 4 years (41), with a mean of 18 years (the longest reported latency was 26 years), and always in people whose exposition began before 1969, when protective measures were still insufficient.

Conclusion

Animal studies allowed the demonstration of VCM toxicity and demonstrated that its metabolites had a genotoxic effect on hepatocyte and sinusoidal cells. Prolonged exposition to VCM (> 4 years in man) with high doses (undetermined in man, and of more than 50 ppm/day in animals) may induce the formation of an endothelial tumor (most often an angiosarcoma) and, by far more rarely, also of a parenchymal tumor (hepatocellular or cholangiocellular carcinoma). After exposition to VCM, these tumors may develop on non-cirrhotic livers.

The latency period for hepatocarcinoma (15 to 44 years after VCM exposition) seems longer than the one for angiosarcoma (9 to 35 years) ; however these two tumors may appear simultaneously.

In animals, the VCM dose necessary to induce a hepatocarcinoma (> 250 ppm) was higher than the one needed to provoke an angiosarcoma (> 50 ppm). Hepatocarcinoma is 10 times less frequent than angiosarcoma. The doses inducing tumors in laboratory animals are very elevated when compared to the exposition doses reported in VCM workers (23,25). In human beings exposed to high VCM doses (data are generally too scarse to measure precisely the level of exposition), some elements are of importance for occurrence of hepatocarcinoma :

In the absence of cirrhosis and of chronic carriage of hepatitis B and C viruses, the occurrence of a hepatocarcinoma may be attributed to VCM. The presence of histological lesions characteristic of VCM damaging effect in the non-tumoral liver is in favor of this relation between VCM and hepatocarcinoma.

If a cirrhosis exists, without markers for chronic infection by hepatitis B or C viruses, without elements suggestive of alcoholic liver disease, in the absence of other diseases leading to cirrhosis, the link between VCM and hepatocarcinoma is more difficult to establish. However, cirrhosis itself may be possibly result from the toxic effect of VCM on the liver, as it is the case for arsenic, thorium dioxyde and anabolic steroids and may then lead to the formation of a hepatocarcinoma.

In a VCM worker who is suffering from alcoholic or viral cirrhosis, the occurrence of a hepatocarcinoma is more often related to alcohol or virus than to VCM toxicity, except if the typical hepatic lesions of VCM toxicity are found in the peritumoral liver.

In man, it is impossible, at least nowadays, to ascertain a synergistic effect between VCM and alcohol or hepatotropic viruses in the pathogenesis of a hepatocarcinoma, even if a concomitant action is possible through the common pathway of P450 cytochrome induction (16,88).

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