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Carcinogenicity Studies on Vinylidene Chloride

by P. L. Viola* and A. Caputo*

Results of a study on Wistar rats regarding a possible carcinogenic effect of vinylidene chloride (VDC) were presented previously by one of the authors.

Due to the unclear character of these results, a second inhalation study was initiated with Sprague-Dawley rats. Groups of 60 animals were exposed to 100 and 75 ppm VDC, along with one control group. A final report on the pathological results must await completion of the microscopic examination of the tissues and organs from all the animals. Nevertheless, it seems clear that there is no grossly observable interrelation between tumor production and VDC inhalation.

As in the case of vinyl chloride, the problem of vinylidene chloride (VDC) is of primary importance in the field of environmental health research. Vinylidene chloride produces no clear signs of toxicity apart from a reduced growth rate observed in some animal species by Prendergast et al. (*1*) and the production of several types of liver damage after 3 months exposure (24 hr/day to 47 ppm). At VDC concentrations of 500 ppm, Cage (*2*) reported that rats exposed to 6 hr/day for 20 days had nose irritation, retarded growth rate, and histologically proven liver cell degeneration. This lesion disappeared when the concentration was lowered to 200 ppm. At this lower concentration a complete autopsy found all organs normal, while in the living animals only a slight nose irritation was observed. Carpenter et al. (*3*) reported that the LC_{50} of VDC is 32,000 ppm in Sherman rats repeatedly exposed for 4 hr/day.

No effects were seen by Norris in rats exposed from 10 to 40 ppm VDC vapors for 30 days (*4*). The only significant finding in Sprague-Dawley rats exposed for 30 or 90 days to 25 or 75 ppm was a minimal vacuolation of cytoplasm in hepatocytes. This lesion reversed when the animals were removed from VDC exposure. Subsequent interim kills at 6 and 12 months confirmed the occurrence of minimal liver injury.

*Regina Elena Institute for Cancer Research, Rome, Italy.

In the 1977 MCA study (*5*), Norris reported that gross pathological examinations of rats exposed to VDC in inhalation chambers showed no compound-related changes in liver, urinary system, respiratory system, or lymphoreticular system.

In a very preliminary account given at the XIth International Cancer Congress (*6*) one of us (P.L.V.) discussed the possible carcinogenic role of VDC by examining the data of a single experiment. In 1975 a systematic investigation of the potential carcinogenic action of VDC was launched.

The inhalation system was similar to that reported in a previous experiment (*7*). Inhalation was performed with VDC purchased from Fluka, purity 99.8%, and thus was assumed to contain no significant amounts of various carcinogenic contaminants. Since VDC is liquid at room temperature, it was necessary to heat it to its boiling point (31.9°C/766 mm Hg). The VDC vapor was collected in a reservoir maintained at the same temperature, and was mixed with a constant amount of air at 32°C in order to reach the desired concentration. This continuously generated atmosphere, was passed throughout the exposure chambers, according to the experimental design reported in the case of the vinyl chloride experiments (*7*)

In the first experiment, from July 1973 to July 1975, 74 male and female, 2-month-old albino Wistar rats were submitted to VDC inhalation for 4 hr/day, 5 days/week for 12 months. Until the end of the fifth month, the VDC concentration was kept at 200 ppm, from the sixth month on it was lowered to

100 ppm to avoid toxic reactions occurring during prolonged exposure. Experimental animals were checked and weighed weekly; those developing poorly were isolated in separate cages. The animals were allowed to die spontaneously or were killed when moribund. The total life span of the experimental rats ranged from 22 to 24 months, and was therefore shorter than those kept as control and maintained under the same conditions, except for the inhalation of VDC. Complete autopsies were performed on all experimental and control animals.

Around the tenth month of inhalation, the occurrence of hard masses next to the external ear ducts was frequently observed. The rapid growing masses became ulcerated and discharged necrotic debris. Biopsies at different times indicated the presence of chronic inflammatory reactions but neoplastic transformation of dermal and/or epidermal tissues was never observed.

The results of this first experiment are reported in Table 1. Tumors are in the abdominal cavity except for one in the liver and one in the lung. Almost all the tumors appeared as large and compact masses located in the middle or lower part of the abdomen and displacing or destroying the intestines. The prominent histological polymorphism and the poor degree of differentiation of these cells indicated that the prevailing components were stem-cells, primitive reticulum cells, for merely undifferentiated mesenchymal cells. Because of these properties and the occurrence of a loose meshwork of reticulin argyrophilic fibrils, the diagnosis of "reticulum cell sarcomas of a nonsincytial type" seems the most appropriate.

Table 1. Tumor incidence in Wistar rats inhaling VDC.

Number of rats	Sex	Number of tumor-bearing animals	Sites of primary tumors: Abdominal cavity	Lung	Liver	% of total
51	M	8	7	—	1	16
23	F	9	8	1	—	39

As reported in Table 2, many abdominal tumors were also found in control rats. The higher incidence of tumors in female rats was confirmed. Microscopically, the tumors were essentially similar in both the VDC inhaling and control groups.

The second study, which is far enough along to be reported, concerned the use of Sprague-Dawley rat, which seemed of interest in view of its different toxicological sensitivity. This inhalation study was started in the summer of 1975. Rats were exposed to VDC concentrations of 100 and 75 ppm VDC, along with one series of controls. As reported in Table 3, the gross pathological examinations of the dead animals and those sacrificed in moribund conditions indicated that in the control group the incidence of tumors was practically identical to that found in the group inhaling 100 ppm VDC and was higher than that observed in the group inhaling 75 ppm VDC.

Table 2. Tumor incidence in control Wistar rats.

Number of rats	Sex	Number of tumor bearing animals	Sites of primary tumors: Abdominal cavity	Lung	Liver	% of total
30	M	5	5	—	—	17
30	F	10	9	1	—	33

Table 3. Incidence of tumors in Sprague-Dawley rats.

Number of rats	Sex	Treatment	Number of tumor-bearing animals	Types of tumors	% of total
30	M	VDC inhalation, 100 ppm	3	Abdominal lymphomas	10
30	F	VDC inhalation, 100 ppm	17	Subcutaneous fibromas(16) Abdominal lymphoma(1)	57
16	M	VDC inhalation, 75 ppm	1	Subcutaneous fibroma	6
21	F	VDC inhalation, 75 ppm	9	Subcutaneous fibromas	43
30	M	None (controls)	—	—	—
30	F	None	15	Subcutaneous fibromas	50

A final statement of the pathological results must await the completion of the microscopic examinations of the tissues and organs of all animals. Nevertheless, it seems clear that there is no grossly observable correlation between tumor formation and VDC inhalation.

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