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Long-Term Carcinogenicity Bioassay on Vinylidene Chloride Administered by Inhalation to Sprague-Dawley Rats^a

New Results

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INTRODUCTION

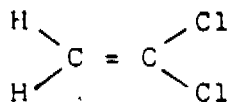
Vinylidene chloride is a monomer mainly used for the production of copolymers and widely employed for food packaging.

The aim of this report is to present recent new results of a long-term carcinogenicity bioassay of vinylidene chloride on rats.

The bioassay was performed at the Bentivoglio (BT) Experimental Unit of the Bologna Institute of Oncology. The experiment was started in 1980 and the biophase ended in 1983.

VINYLIDENE CHLORIDE

Vinylidene chloride (CH_2CCl_2) has a molecular weight of 97.0 and its structural formula is



Data on the production, uses, and occurrence of the compound are given in TABLES 1, 2, and 3. The findings in the literature on carcinogenicity and other relevant biological effects are shown in TABLE 4. They conclude that:

(1) *in rats* treated by inhalation, there was limited evidence of an increased incidence of mammary tumors (not dose-related), and possibly of liver and meningeal tumors;

(2) *in mice* vinylidene chloride is carcinogenic, causing kidney adenocarcinomas, and an increased incidence of lung tumors, mammary carcinomas, and malignant tumors. In vinylidene-treated mice, limited evidence was found of an increased incidence of lymphomas and leukemias in females that was not dose-

^a This experiment was partly supported by the EEC (Contract 323-79-4-ENV-1).

TABLE 1. Vinylidene Chloride: Production

<i>Methods of Production</i>
Vinylidene chloride may be prepared by several methods. Most of the monomer (USA, Japan) is produced commercially by the dehydrochlorination (using sodium and hydroxide or lime) of 1,1,2-trichloroethane
<i>World Production</i>
130,000 tons per year
<i>Geographical Areas of Production</i>
USA (70,000 tons, 1976), Western Europe (30,000 tons), Japan (30,000 tons)

TABLE 2. Vinylidene Chloride: Uses

<i>Major Uses</i>
Production of copolymers with high vinylidene chloride content (the other major monomer used is vinyl chloride), used in the form of film coatings (solvent soluble resins and latexes) and fibers, and for tubes, pipes and pipe liners
<i>Other Uses</i>
Manufacture of modacrylic fibers (largely based on acrylonitrile); as a chemical intermediate in the production of 1,1,1-trichloroethane

TABLE 3. Vinylidene Chloride: Occurrence^a

<i>Natural Sources</i>
It is not known to occur as a natural product
<i>Workplaces</i>
Where vinylidene chloride or products containing vinylidene chloride are produced, used, and stocked
<i>Air</i>
Atmosphere of submarines Spacecraft
<i>Water</i>
Effluents discharged from chemical manufacturing plants Rivers Wells Other untreated water Finished USA drinking water (tap water)
<i>Food</i>
Potato chips packed in polypropylene film coated with vinylidene chloride copolymers
<i>Industrial Materials</i>
In trichloroethylene, vinyl chloride and commercial chloroprene Film for packaging

^a From IARC¹ and Maltoni *et al.*²

TABLE 4. Vinylidene Chloride: Data on Carcinogenicity and Other Relevant Correlated Biological Effects

(1) Carcinogenicity Studies

(A) Experimental animals

(a) Rat: by inhalation

- limited evidence of increased incidence of mammary tumors in female Sprague-Dawley rats²⁻⁴
- no effects on Sprague-Dawley and Wistar rats⁴
- no effects on Sprague-Dawley rats⁶
- limited evidence of induction of extrahepatic angiosarcomas in CD rats.^{7,8} Concurrent unwanted exposure to vinyl chloride cannot be ruled out in this study²
- no effects on CD rats⁹

Rat: by ingestion

- (stomach tube): no effects on Sprague-Dawley rats²⁻⁴
- (drinking water): no effects on Sprague-Dawley rats⁶
- (stomach tube): limited evidence of increased incidence of liver and meningeal tumors in BDIV rats¹⁰
- (stomach tube): no effects on F344/N rats¹¹

(b) Mouse: by inhalation

- in Swiss mice, induction of kidney adenocarcinomas in males, and increased incidence of mammary carcinomas, lung tumors and total malignant tumors in females²⁻⁴
- induction of liver angiosarcomas and increased incidence of lung adenomas in CD-1 mice.^{7,8} Concurrent unwanted exposure to vinyl chloride cannot be ruled out in this study²
- no effects in CD-1 mice⁹

Mouse: by ingestion

- (stomach tube): limited evidence of increased incidence of lymphomas and leukemias in female B6C3F1 mice¹¹

Mouse: by skin painting

- skin tumor initiator on Ha:ICR Swiss mice¹²

(c) Hamster: by inhalation

- no effects on Chinese hamsters²⁻⁴

(B) Humans: occupational exposure

- no evidence of an increased number of malignant tumors.^{13,14} These studies cannot be considered conclusive

(2) Genetic Effects

(A) Bacterial systems:

- mutagen after metabolic activation

(B) Other systems:

- increase in the chromosome aberration rate in Chinese hamsters, following inhalation and ingestion exposure¹⁵

related. Vinylidene chloride was also shown to be an initiator of skin carcinogenesis.

(3) In Chinese hamsters no carcinogenic effects of the monomer were found.

The reported results in rats do not conclusively prove that vinylidene chloride is carcinogenic in this animal species. Therefore, until the results of the experiment herewith reported were obtained, the evidence for carcinogenicity of vinylidene chloride was almost exclusively drawn from the data of the bioassays in mice.

her Relevant

LONG-TERM CARCINOGENICITY BIOASSAY PERFORMED AT THE BENTIVOGLIO (BT) EXPERIMENTAL UNIT OF THE BOLOGNA INSTITUTE OF ONCOLOGY

Materials, Methods, Plan, and Conduct of the Experiment

n female Sprague-

Vinylidene chloride was tested in Sprague-Dawley rats by inhalation. Details on the test material and test animals are given in TABLE 5.

n CD rats.^{1,2} Con-
out in this study²

The chambers for inhalation exposure were made of stainless steel, with two glass doors, and they measure 135 × 98 × 65 cm. The volume of each chamber is 860 liters. Continuous air flow provided 12-15 air changes per hour. Before its introduction the air was filtered, and the chamber arrangement was such that air flowed from one part of the chamber to the other without recirculation. The internal pressure was about 1 mm Hg less than that of the room where the chambers were situated to avoid any possible contamination of the outside environment. The concentrations in air were checked by continuous gas chromatographic monitoring.

er and meningeal

The plan of the experiment is shown in TABLE 6, while details on the conduct of the experiment are presented in TABLE 7.

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Systematic and standardized histopathologic examinations were performed in each animal on the subcutaneous lymph nodes, brain and cerebellum, Zymbal glands, interscapular brown fat, salivary glands, Harderian glands, tongue, thymus and mediastinal lymph nodes, lungs, diaphragm, liver, kidneys, adrenals, spleen, pancreas, esophagus, mesenteric lymph nodes, stomach, various segments of the intestine, bladder, uterus, gonads, sternum, and any other organ with pathologic lesions.

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: cannot be ruled

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Results

Survival. The treatment with vinylidene chloride did not affect the survival rate of any of the exposed groups.

These studies

TABLE 5. Vinylidene Chloride: Test Compound and Test Animals

Test Compound

(A) *Supplier*: Solvay, Belgium

(B) *Purity*:

Vinylidene chloride

999.43 g/kg

1,2-dichloroethylene

0.40 g/kg

acetone

0.10 g/kg

methylene chloride

0.05 g/kg

mono- and dichloroethylene

0.02 g/kg

paramethoxyphenol

200 ppm

(as stabilizer)

Test Animals

Male and female Sprague-Dawley rats, breeders 13 weeks old and embryos (12th day of gestation) at the start of experiment.

The animals were of the breed currently used in the BT Experimental Unit for more than 15 years.

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TABLE 6. Vinylidene Chloride: Plan of the Experiment and Inhalation Schedule in Sprague-Dawley Rats (Exp. BT 4002, 4006)

Group No.	Concentration (ppm)	Schedule	Animals		
			Age	Sex	No. at Start
I	100	4 hd/5 dw/7 w ^a and then 7 hd/5 dw/97 w ^b	Breeders	F	54
II	0 (Control)	—	Breeders	F	60
I	100	4 hd/5 dw/7 w and then 7 hd/5 dw/97 w	Embryos	M	62
				F	61
				M + F	123
II	100	4 hd/5 dw/7 w and then 7 hd/5 dw/8 w ^c	Embryos	M	60
				F	60
				M + F	120
III	0 (Control)	—	Embryos	M	158
				F	149
				M + F	307
Total				M	280
				F	384
				M + F	664

^a Four hours a day for 5 days a week for 7 weeks.

^b Seven hours a day for 5 days a week for 97 weeks.

^c Seven hours a day for 5 days a week for 8 weeks.

Body weight. In breeders and in male and female offspring treated with vinylidene chloride for 104 weeks, a slight decrease in body weight was observed. In offspring treated for 15 weeks, a slight body weight decrease was evident in male but not in female animals.

Carcinogenicity. In rats of the strain used, the tumors most frequently expected on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary tumors (benign and malignant), leukemias, pheochromocytomas and pheochromoblastomas. Moreover, a variety of other miscellaneous tumors are also observed.^{16,17}

TABLE 7. Vinylidene Chloride: Conduct of the Experiment

- The animals were exposed by:
Inhalation, 4-7 hours daily, 5 days weekly, for 104 weeks (breeders)
Transplacental route and then inhalation, 4-7 hours daily, 5 days weekly, for 104 weeks or 15 weeks (12-day embryos)
- All the animals were kept under observation until spontaneous death.
- The status and behavior of the animals were examined 3 times daily.
- The animals were submitted to clinical examination for gross changes every 2 weeks.
- The animals were weighed every 2 weeks during treatment and then every 8 weeks.
- Full necropsy and histopathologic examination were performed on all the animals.
- The housing and the diet of the animals were the same highly standardized ones adopted in the BT Experimental Unit during the last 15 years

TABLE 8. Vinylidene Chloride: Total Tumors in Rats (Exp. BT 4002, 4006)

Group No	Concentration (ppm)	Schedule	Animals			Percentage of Animals Bearing Tumors		No. of Malignant Tumors per 100 Animals
			Age	Sex	No. at Start	TBMT*	MT†	
I	100	4 hd/5 dw/7 w ^c and then 7 hd/5 dw/97 w ^d	Breeders	F	54	68.5	20.4	22.2
II	0 (Control)	—	Breeders	F	60	58.3	15.0	16.7
I	100	4 hd/5 dw/7 w and then 7 hd/5 dw/97 w	Embryos	M	62	62.9	32.2	33.9
				F	61	67.2	29.5	34.4
				M + F	123	65.0	30.9	34.1
II	100	4 hd/5 dw/7 w and then 7 hd/5 dw/8 w ^e	Embryos	M	60	58.3	21.7	23.3
				F	60	78.3	23.3	23.3
				M + F	120	68.3	22.5	23.3
III	0 (Control)	—	Embryos	M	158	44.9	17.1	18.3
				F	149	78.5	17.4	17.4
				M + F	307	61.2	17.3	17.9

* Total benign and malignant tumors.

† Malignant tumors.

^c Four hours a day for 5 days a week for 7 weeks.^d Seven hours a day for 5 days a week for 97 weeks.^e Seven hours a day for 5 days a week for 8 weeks.

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TABLE 9. Vinylidene Chloride: Mammary Tumors, Leukemias, Pheochromocytomas and Pheochromoblastomas in Rats (Exp. BT 4002, 4006)

Group No.	Concentration (ppm)	Schedule	Animals			Percentage of Animals Bearing Tumors				
			Age	Sex	No. at Start	Mammary Tumors		Leukemias ^c	Pheochromocytomas	Pheochromoblastomas
I	100	4 hd/5 dw/7 w ^d and then 7 hd/5 dw/97 w ^e	Breeders	F	54	53.7	7.4	1.8	7.4	1.8
II	0 (Control)	—	Breeders	F	60	40.0	3.3	3.3	18.3	—
I	100	4 hd/5 dw/ 7w and then 7 hd/5 dw/97 w	Embryos	M	62	11.3	—	16.1	30.6	—
				F	61	45.9	4.9	6.5	4.9	—
				M + F	123	28.4	2.4	11.4	17.9	—
II	100	4 hd/5 dw/7 w and then 7 hd/5 dw/8 w ^f	Embryos	M	60	5.0	—	13.3	28.3	1.7
				F	60	53.3	10.0	3.3	11.7	—
				M + F	120	29.2	5.0	8.3	20.0	0.8
III	0 (Control)	—	Embryos	M	158	7.0	1.9	7.6	22.8	0.6
				F	149	56.4	5.4	0.7	18.8	0.7
				M + F	307	30.9	3.6	4.2	20.8	0.6

^a Benign and malignant tumors.

^b Malignant tumors.

^c The term "leukemia" includes a variety of hemolymphoreticular neoplastic diseases at different sites.

^d Four hours a day for 5 days a week for 7 weeks.

^e Seven hours a day for 5 days a week for 97 weeks.

^f Seven hours a day for 5 days a week for 8 weeks.

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An increased percentage of rats bearing malignant tumors and an increased number of malignant tumors per 100 animals were found in offspring of both sexes treated with vinylidene chloride for 104 weeks. There was only limited evidence of a borderline increase in total malignant tumors in the other exposed groups, i.e., offspring treated for 15 weeks and breeders treated for 104 weeks (TABLE 8).

An increased incidence of "leukemias" was found in the vinylidene-chloride-exposed offspring; this increase was related to the length of treatment (TABLE 9).

No relevant differences were observed in the incidence of other tumors frequently occurring in untreated rats of the BT colony (TABLE 9). No unexpected tumors related to treatment were found.

CONCLUSIONS

These results demonstrate that under particular experimental conditions (i.e., prolonged exposure started during embryonal development, when responsiveness to carcinogenic agents is usually higher), vinylidene chloride has carcinogenic effects on rats, too.

These effects, in our bioassay, were shown by the increased incidence of malignant tumors and leukemias among the exposed animals.

SUMMARY

Vinylidene chloride was administered by inhalation, 7 hours daily, 5 days weekly, at the concentration of 100 and 0 ppm, to Sprague-Dawley rats. The treatment was started on 13-week-old breeders, and male and female offspring (12-day embryos). The breeders and some of the offspring were exposed for 104 weeks; the other offspring were exposed for 15 weeks only.

An increased incidence was found of malignant tumors and of leukemias, particularly in offspring treated for 104 weeks.

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