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VINYL CHLORIDE: A MODEL CARCINOGEN FOR RISK ASSESSMENT

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INTRODUCTION

In 1971, an extensive, integrated program of long term studies on carcinogenicity was initiated at the Institute of Oncology and Tumor Center of Bologna. The main objectives of the program were: to test the carcinogenic potency of several major airborne industrial compounds; to obtain information on the site and type of tumors induced by the compounds; to assess the level of risk in quantitative terms through dose response data; to determine the relative potency of the different compounds; to evaluate the effects of route of administration on tumor type and incidence; and to determine the role of different biological factors such as species, strain, sex and age on tumor yields.

Since 1971, several compounds have been tested by one or more routes, in one or more species and strains and at different concentrations. These agents include: vinyl chloride, vinylidene chloride, ethylene dichloride, trichloroethylene, methylene chloride, acrylonitrile, propylene, benzene, styrene, trichlorofluormethane, dichlorodifluormethane and chlorodifluoromethane. Vinyl chloride (VC) has been the industrial agent of greatest interest since it represents a successful attempt to assess risk to man by experiments in vinyl chloride carcinogenicity in animals.

MATERIALS AND METHODS

The animals are breeds (Sprague-Dawley rats, Wistar rats, Swiss mice, golden hamsters) which have been routinely employed in our laboratory for many years. The animals are weighed every two weeks during treatment and at least every eight weeks after the end of the treatment. Every two weeks, the animals are examined for any gross changes. All animals are kept alive until spontaneous death. A full autopsy is performed on each animal. Specimens taken for routine histology include: brain, Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, adrenals, spleen, pancreas, stomach, intestine, uterus, gonads and any other organ with an observable pathological lesion. Sections from prepared specimens are routinely stained with Hematoxylin-Eosin. All slides are screened by a junior pathologist and then reviewed by a senior pathologist.

Our inhalation facilities permit the simultaneous exposure of 6,000 laboratory rodents (rats, mice and hamsters). The inhalation chambers are built of stainless steel and glass. An automatic gas chromatography system is used to monitor the exposure level in the inhalation experiments. For ingestion studies, the test compound is administered by gavage, mixed with food or dissolved in drinking water. All test compounds and carrier material are routinely examined to determine that they meet purity standards. Extra-virgin olive oil is used as the carrier in gavage studies. Each treatment is performed by the same individual; this is particularly important in gavage treatments, since the animals become accustomed to the same operator.

All data are submitted to statistical analysis. However, we must stress that although statistical analysis provides an extremely important tool for interpreting the meaning of the results of long-term bioassays, there may be nonstatistically significant differences between exposed and control groups which could still be meaningful from an oncological point of view. This is particularly true in the case of tumors which occur only rarely in our animal colony. Therefore, the data are evaluated both from statistical and from biological perspectives.

RESULTS

Vinyl chloride has been tested on animals of different species, strain, sex and age. The choice of the animals was made with the intention of having an integrated system of complementary biological models which could express a wide range of neoplastic reponses. VC was administered by four different routes (inhalation, ingestion, intraperitoneal and subcutaneous). The

monomer was administered at different concentrations (fifteen by inhalation, six by ingestion, three by intraperitoneal injections and one by subcutaneous injection), for various periods of time, by continuous or intermittent treatment.

Eighteen experiments were initiated, seventeen experiments have ended and have been evaluated and one experiment is still ongoing. A total of 6,680 animals were used in these studies.

The ability of VC to induce tumors was determined on the basis of one or more of the following parameters: (a) a sharply enhanced tumor incidence; (b) rare or exceptional tumor occurrence in the animal colony; (c) a dose-response relationship for tumor formation; and (d) an association with precarcinogenic lesions.

The following conclusions can be drawn from the data produced in the seventeen VC experiments. Exposure to VC caused tumors in all animal systems. The distribution of different tumor types in different sites, e.g. Zymbal gland carcinoma, liver angiosarcoma, nephroblastoma, neuroblastoma, mammary gland carcinoma and forestomach papilloma, etc., clearly demonstrated that VC is a multipotential carcinogen (Table 1). Some of the same types of tumors were observed in all of the animal species studied, e.g. liver angiosarcomas, while other tumors, e.g. nephroblastoma, were observed in only one animal system. VC induced a dose-response increase in tumors when given by inhalation or ingestion (Table 2). The duration and schedule for the VC inhalation treatment greatly affected the neoplastic response (Table 3). Qualitatively and quantitatively, the neoplastic response to inhaled VC is greatly modulated by the species, e.g., for liver angiosarcoma--Swiss mice > rats > Golden hamsters (Table 4); the strain, e.g. for Zymbal gland carcinoma--Sprague-Dawley > Wistar (Table 5); the age, e.g. for liver angiosarcoma--newborn > 11 week old rats (Table 6); and the sex, e.g. for liver angiosarcoma--female rats > male rats (Table 2 for example). Newborn animals appeared to be extremely responsive to the carcinogenic ability of VC by easily developing liver tumors; not only angiosarcomas but also hepatocarcinomas. Exposure of pregnant animals to VC was also carcinogenic in embryos.

The results of the experiments on Sprague-Dawley rats initiated to study the effects of different doses of VC, when given by inhalation and by ingestion, were submitted to statistical analysis by the Fisher exact probability test ($p < 0.05$). The concentration of VC inducing a significant number of excess cancer cases in these experiments are given in Table 7.

In our opinion, the following results, although not statistically significant according to the Fisher exact probability test should be specifically noted as biologically of importance.

Table 1. Vinyl chloride: tumors on experimental rodents presently correlated to the monomers.

Species	Angiosarcomas of liver	Tumors of brain	Tumors of lung	Lymphomas and leukemias	Hepatomas	Angiosarcomas angiomas of other sites
Rat	+	+			+	+
Mouse	+		+			+
Hamster	+			(+)		(+)

Species	Nephrobastomas	Sebaceous cutaneous carcinomas	Other epithelial tumors	Mammary Carcinomas	Forestomach papillmoas and acanthomas	Melanomas
Rat	+	+	(+)	+	+	
Mouse			(+)	+	(+)	
Hamster			(+)		+	(+)

\+ statistically significant increase
(+) meaningful increase

Table 2A. Incidence (%) of Zymbal gland carcinomas in Sprague-Dawley rats after exposure to vinyl chloride: inhalation vs ingestion

Inhalation*

Concentration (ppm)	% Animals with Tumors Male	Female	Total
30,000	56.6	60.0	58.3
10,000	33.3	20.0	26.7
6,000	10.3	13.3	11.9
2,500	3.3	3.3	3.3
500	10.0	3.3	6.7
250	-	-	-
200	5.0	1.7	3.3
150	-	6.7	3.4
100	-	1.7	0.8
50	2.3	2.8	2.5
25	5.0	1.7	3.3
10	1.7	1.7	1.7
5	-	1.7	0.8
1	1.7	-	0.8
0	0.9	0.8	0.9

*4 hr/d, 5 d/wk, 52 wk; 2025 animals total, 60-360 animals per group

Ingestion**

Concentration (mg/Kg b.w.)	% Animals with Tumors Male	Female	Total
50.00	2.5	-	1.2
16.65	2.5	2.5	2.5
3.33	-	-	-
1.0	2.7	4.0	3.3
0.3	-	-	-
0.03	-	-	-
0	-	1.7	0.9

**Gavage: 5 times/wk, 52 (or 59) wk; 920 animals total, 80 or 150 animals per group.

Table 2B. Incidence (%) of liver angiosarcomas in Sprague-Dawley rats after exposure to vinyl chloride: inhalation vs ingestion

Inhalation*

Concentration (ppm)	% Animals with Tumors Male	Female	Total
30,000	16.6	43.3	30.0
10,000	10.0	13.3	11.7
6,000	10.3	33.3	22.0
2,500	20.0	23.3	21.7
500	-	20.0	10.0
250	3.4	6.7	5.1
200	11.7	8.3	10.0
150	1.7	8.3	5.0
100	-	1.7	0.8
50	1.1	7.2	4.2
25	1.7	6.7	4.2
10	-	1.7	0.8
5	-	-	-
1	-	-	-
0	-	-	-

*4 hr/d, 5 d/wk, 52 wk; 2025 animals total, 60-360 animals per group.

Ingestion**

Concentration (mg/Kg b.w.)	% Animals with Tumors Male	Female	Total
50.00	20.0	22.5	21.2
16.65	10.0	15.0	12.5
3.33	-	-	-
1.0	1.3	2.7	2.0
0.3	-	1.4	0.7
0.03	-	-	-
0	-	-	-

**Gavage; 5 times/wk, 52 (or 59) wk; 920 animals total,; 80 or 150 animals per group.

Table 3. Incidence (%) of liver angiosarcomas and Zymbal gland carcinomas in Sprague-Dawley rats after vinyl chloride inhalation: effect of exposure regimen

Conc. (ppm)	Schedule*	% Animals with Tumors** liver angiosarcomas M	F	T	Zymbal gl. ca. M	F	T
10,000	I	10.0	13.3	11.7	33.3	20.0	26.7
10,000	II	-	-	-	17.8	13.3	15.5
10,000	III	1.7	-	0.8	13.5	1.7	7.6
10,000	IV	1.7	-	0.8	8.5	6.7	7.6
10,000	V	-	1.7	0.8	3.3	10.2	6.7
6,000	I	10.3	33.3	22.0	10.3	13.3	11.9
6,000	II	-	3.3	1.7	20.0	10.0	15.0
6,000	III	-	-	-	10.0	5.0	7.5
6,000	IV	3.4	1.7	2.5	8.5	-	4.2
6,000	V	-	1.7	0.8	10.0	5.0	7.5

*4 hr/d, 5 d/wk, 52 wk (I); 60 animals per group.

4 hr/d, 5 d/wk, 17 wk (II); 60 animals per group.

4 hr/d, 5 d/wk, 5 wk (III); 120 animals per group.

1 hr/d, 4 d/wk, 25 wk (IV); 120 animals per group.

4 hr/d, 1 d/wk, 25 wk (V); 120 animals per group.

**M=male; F=female; T=total.

Table 4. Incidence (%) of liver angiosarcomas after chronic vinyl chloride inhalation: effect of species

Concentration (ppm)	% Male Animals with Tumors			
	Sprague-Dawley Rats[a]	Wistar Rats[b]	Swiss Mice[c]	Golden Hamsters[d]
10,000	10.0	29.6	3.8	-
6,000	10.3	11.5	6.7	3.3
2,500	20.0	12.0	20.7	-
500	-	10.0	20.0	6.7
250	3.4	3.7	30.0	-
50	-	-	3.3	-
0	-	-	-	-

[a] 4 hr/d, 5 d/wk, 52 wk; 60 animals per group.

[b] 4 hr/d, 5 d/wk, 52 wk; 30-40 animals per group.

[c] 4 hr/d, 5 d/wk, 30 wk; 60-150 animals per group.

[d] 4 hr/d, 5 d/wk, 30 wk; 30-62 animals per group.

Table 5. Incidence (%) of Zymbal gland carcinoma after chronic vinyl chloride inhalation: effect of strain

Concentration (ppm)	% Male Animals with Tumors Sprague-Dawley Rats[a]	Wistar Rats[b]
10,000	33.3	7.4
6,000	10.3	7.7
2,500	3.3	-
500	10.0	-
250	-	-
50	-	-
0	-	-

[a] 4 hr/d, 5 d/wk, 52 wk; 60 animals per group.

[b] 4 hr/d, 5 d/wk, 52 wk; 30-40 animals per group.

Table 6. Incidence (%) of liver angiosarcomas in Sprague-Dawley rats after exposure to vinyl chloride by inhalation: effect of age

Concentration[a] (ppm)	% Animals with Tumors* Newborn rats[b,c] M	F	T	11 weeks old rats[b,d] M	F	T
10,000	25.0	45.0	34.1	1.7	-	0.8
6,000	27.8	50.0	40.5	-	-	-

* M=male; F=female; T=total

[a] 4 hr/d, 5 d/wk, 5 wk
[b] Age at start of exposure
[c] 43-45 animals per group
[d] 126 animals per group

Table 7A. Vinyl chloride: concentrations where total cancer bearing Sprague-Dawley rats were significantly in excess*

Sex	Inhalation[a] (ppm)	Ingestion[b] mg/Kg b.w.
Male	30,000; 10,000; 6,000; 2,500; 500; 250; 200; 50	50
Female	30,000; 10,000; 6,000; 2,500; 500; 200; 150; 50	50

*Fisher exact probability test ($p \leq 0.05$)

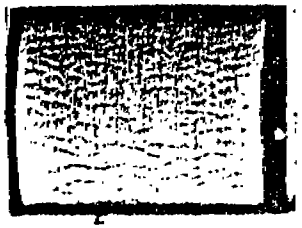
[a]4 hr/d, 5 d/wk, 52 wk; 2025 animals, 60-360 animals per group
[b]Gavage; 5 times/wk, 52 wk; 920 animals, 80 or 150 animals per group

Extra-hepatic angiosarcomas of different sites: These tumors are observed at a very low incidence in untreated Sprague-Dawley rats of our colony. Our data strongly suggest a VC dependency in the appearance of these tumors in these rats. This relationship is supported by the excessive incidence of extra-hepatic vascular tumors in mice treated with VC.

Hepatomas: A few cases of hepatomas have been observed in VC-treated groups of animals, when exposed as adults. This tumor is exceptionally rare in our Sprague-Dawley rat colony, and none have been observed in the control animal groups of the seventeen experiments. Moreover, a high incidence of hepatomas was observed in Sprague-Dawley rats following neonatal exposure to a high concentration of VC for a short period.

Considering their rare occurrence, or total absence, in our animal colony, particular attention should also be given to the appearance of the following tumors, even when not statistically significant in animals exposed to low doses.

Zymbal gland carcinomas: An excess of these tumors appears to be present in Sprague-Dawley at airborne concentrations down to 50 and 25 ppm.



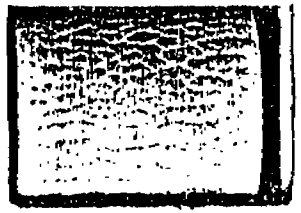


Table 7B. Vinyl chloride: tumors significantly in excess*

Tumor	Sprague-Dawley Rats	Exposure Inhalation[a] (ppm)	Ingestion[b] (mg/Kg b.w.)
Zymbal gland carcinoma	Male	30,000;10,000	
	Female	30,000;10,000	
Liver angiosarcoma	Male	30,000; 2,500; 200	50
	Female	30,000; 6,000; 2,500; 500; 200; 150; 50	50; 16.65
Nephroblastoma	Male	2,500; 200, 150, 100	
	Female	500; 250	
Neuroblastoma	Female	10,000	
Mammary gland adenocarcinoma	Female	150; 50; 25, 10; 5	
Forestomach papilloma	Male	30,000	
	Female	30,000	

*Fisher exact probability test ($p<0.05$)
[a]4 hr/d, 5 d/wk, 52 wk; 2025 animals, 60-360 animals per group.
[b]Gavage; 5 times/wk, 52 wk; 920 animals, 80 or 150 animals per group.

Liver anigosarcomas: This tumor is extremely rare in our animal colony (4 cases in several thousand untreated animals). Therefore, one must consider their appearance as important even when not statistically different. Their appearance at airborne concentrations below 50 ppm (5 liver angiosarcomas out of 120 animals at 25 ppm, and 1 liver angiosarcoma out of 120 animals at 10 ppm), and at ingested doses of 1 mg/Kg b.w. (3 liver angiosarcomas out of 150 animals), and at 0.3 mg/Kg b.w. (1 liver angiosarcoma out of 150 animals) is especially striking.

Nephroblastomas: The onset of a few of these tumors, observed after inhalation treatment at doses below 100 ppm and in groups treated by ingestion with 50 and 16.65 mg/Kg b.w. is not accidental in our opinion, given the extreme rarity of these tumors in rats.

Neuroblastomas: To date, this tumor has never been observed by us in control or otherwise treated Sprague-Dawley rats used in our laboratory. Therefore, we consider their occurrence as dependent on VC treatment even at inhaled exposure levels below 10,000 ppm, e.g. 6,000 and 2,500 ppm.

The ability of low concentrations of VC to induce tumors may be better evaluated by considering all tumor types (Table 8). Below 10 ppm and 0.3 mg/Kg b.w., no increase in the specifically

Table 8. Induction of tumors at low concentrations of vinyl chloride in Sprague-Dawley rats*

at 25 ppm	: over 120 animals; 5 liver angiosarcomas, 4 Zymbal gland carcinomas and 1 nephroblastoma.
at 10 ppm	: over 120 animals; 1 liver angiosarcoma, 2 extra-hepatic angiosarcomas and 2 Zymbal gland carcinomas.
at 1 mg/Kg	: over 150 animals; 3 liver angiosarcomas, 1 extra-hepatic angiosarcoma, 1 hepatoma and 5 Zymbal gland carcinomas.
at 0.3 mg/Kg	: over 150 animals; 1 liver angiosarcoma and 1 hepatoma.

*not significantly different by the Fisher exact probability test ($p \leq 0.05$) but biologically meaningful based on the rare occurrence of the specified tumors in the animal colony.

VC related tumors were observed in the experiments on Sprague-Dawley rats.

CONCLUSION

As outlined historically in Table 9, these experimental studies led to the discovery that VC is carcinogenic and to an approach for quantifying the risk. As a direct consequence, of our investigation considerable effort has been made to control occupational exposure to this industrial carcinogen. Long-term carcinogenicity bioassays on VC were a crucial step in the field of environmental and occupational carcinogenesis which, in turn, are among the most important areas of public health.

The VC project and the other bioassays on industrial compounds of the program being carried out in our Institution have demonstrated that the experimental approach is a unique tool for:

1. identifing environmental and occupational carcinogens;
2. predicting target organs and type of tumors;
3. assessing the risk in quantitative terms in relation to dose;
4. providing a highly standardized model for the classification of compounds by potency; and
5. improving our scientific knowledge aimed at selecting the most suitable experimental protocols for extrapolating animal data to humans.

Table 9. The history of Vinyl Chloride carcinogenicity

1961	: VC was found to produce liver enlargement and microscopic hepatic degenerative changes (1).
1970	: Zymbal gland carcinomas were reported in rats exposed to 30,000 ppm of VC, by inhalation (2).
1970	: An increase in atypias in respiratory cells was observed among workers heavily exposed to VC (3).
1971 (July)	: A vast project of long-term carcinogenicity bioassays on VC was started in Bentivoglio, near Bologna, Italy (the BT Project)

1972 (August) : Zymbal gland carcinomas, nephroblastomas and liver angiosarcomas were observed in rats exposed to VC by inhalation (Maltoni, BT Project)

1973 (April) : The first data of the BT Project were released to the scientific community: the oncogenic effect was observed down to 250 ppm (3).

1973 : Splenomegalic liver disease was found among polyvinyl chloride production workers (4).

1973 (December) : For the first time a case of liver angiosarcoma in a worker of polyvinyl chloride production was correlated to VC exposure (5).

1974 (February) : On the basis of the BT Project data indicating a carcinogenic effect at 250 ppm, OSHA proposed a TLV of 50 ppm (Maltoni: testimony at the I OSHA Hearing on VC, 1974).

1974 (February) : The BT project data showed that VC is a multi-potential carcinogen, producing a variety of tumors, in different animal species (Maltoni: testimony at the I OSHA Hearing on VC, 1974).

1974 : The BT project data indicated a carcinogenic effect at 50 ppm. OSHA proposed new stricter rules (Maltoni: communication at the II OSHA Hearing on VC, 1974; 6).

1974 : Early epidemiological observation (paralleling the experimental information) indicated an increase in tumors other than liver angiosarcomas (of brain, lung, liver, hemolymphoreticular tissues) among workers of VC-PVC industries (7).

1974 : BT project data showed that VC had carcinogenic effects in rats also when given by ingestion (8).

1976 : In rats of the BT project exposed to VC by inhalation, angiosarcomas were observed down to the level of 25 ppm, and Zymbal gland carcinomas down to the level of 10 ppm (9). (These data were unaccountably omitted in IARC Monograph 19, February 1979).

1979 : Presentation of the final results of the effects of long-term exposure to a range of 14 doses by inhalation (from 30,000 to 1 ppm) and of 6 doses by ingestion (from 50 mg to 0.03 mg/Kg b.w.) on Sprague-Dawley Rats, at the XXe Reunion du Club de Cancerogenese Chimique, Fondation Curie, Paris" (10).

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DISCUSSION

Q. Infante (U.S. Dept. of Labor): Given the same species, how much difference in carcinogenic response do you see by the various routes of administration?

A. Maltoni (Italy): I would say that the ratio of certain cancer types is very much changed by ingestion.

Q. Infante (U.S. Dept. of Labor): Rather than incidence, how about in terms of site-specific cancer induction?

A. Maltoni (Italy): I would say that by inhalation for vinyl chloride we get a broader spectrum of tumors.