#249

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VINYL CHLORIDE CARCINOGENESIS: CURRENT RESULTS AND PERSPECTIVES

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I) Introduction

Vinyl chloride (VC) has been produced industrially for more than 30 years in many countries, such as the U.S.A., Japan, Western and Eastern Europe, Russia, Canada, South Africa, Australia, the Philipines and Brazil. VC is the constituent of polyvinyl chloride (PVC), which today is the plastic industrial material most often used and diffused throughout the world. It is used as a copolymer in Saran and other plastics, as a chemical intermediate, as a solvent, and as a propellant; in the past, for only a short period, it was also used as an anaesthetic.

Some sectors of industry and modern life, heavily dependent on VC and PVC products, include building and construction, the automobile industry, electrical wires and cables, industrial and household equipment, packaging products, household furnishings and medical products. Other polymers containing vinyl chloride are used in fibres and surface coatings. VC monomer is the single most important market for the chloride-caustic industry. The rubber, paper and glass industries also depend heavily on the production of VC.

VC is presently produced at a world-wide rate of approximately 12 million tons per year, meaning the entire VC-PVC industry has a substantial impact on the world's economy.

For instance, in just the U.S., which produced more than ⅓ of the world's VC, approximately 14,000 workers are employed in nearly 50 monomer and resin plants, and an estimated 50,000 workers are employed in plants processing PVC into numerous products, which in turn are used by an estimated 800,000 to 900,000 more workers to produce industrial goods and individual consumer products.

The economic value in the U.S. of operations dependent upon VC-PVC production is measured in many billions of dollars. The *Washington Post* reported on June 26, 1974 the following: "The American plastics industry warned yesterday that proposed government rules to slash vinyl chloride levels in factories to eliminate the danger of cancer could cost the nation up to 2.2 million jobs and as much as 90 billions U.S. dollars in annual production."

In Italy the production of VC is evaluated at approximately 720,000 tons per year. The major producing companies of VC/PVC are: Montedison, Solvay, Ferronica, ANIC and Liquichimica.

The following population groups may be exposed to VC: 1) workers engaged in the production of PVC and VC, and the use of VC for other industrial purposes; 2) workers manufacturing PVC; 3) residents in areas neighbouring factories producing PVC and VC; and to a much lesser extent: 4) people using products containing VC, such as some propellent sprays; 5) people having contact with resins made with VC and with materials containing these resins; and 6) populations consuming food contained in plastics made by VC.

Bioassays on the long term effects of VC were not available until 1970. This situation reflects the lack of consideration given until now to experimental bioassays in predicting the oncogenic effect on man of environmental and occupational agents. We believe that the events related to vinyl chloride carcinogenicity should induce greater interest in experimental bioassays.

II) History

In 1970 Professor Viola presented (Viola, 1970) at the X International Cancer Congress (Houston) the results of a pilot experiment, which were later published in detail (Viola et al., 1971). Professor Viola reported to have observed in 26 rats exposed by the inhalatory route to 30,000 ppm of VC 4 hours daily, 5 days weekly, for 10-12 months, surviving 10 months or more, 6 skin tumours, 7 pulmonary tumours, mainly carcinomas, and 5 osteochondromas.

In 1967 our Institute initiated a large investigation among workers of several Italian industrial plants to determine the effects of the exposure to different chemicals on respiratory epithelia. The pathological changes were studied by sputum cytology. In 1970 this research was extended to workers in VC/PVC industries. A significant increase of cellular changes in bronchial epithelium, such as marked squamous metaplasia and squamous dysplasia, was found among the workers more heavily exposed to VC.

Immediately after the Houston Congress, we contacted Professor Viola who finally put some slides of his experimental tumours at our disposal. According to our interpretation, the malignant tumours observed by Professor Viola in the skin were carcinomas arising from the Zymbal gland (a sebaceous gland of the exterior acoustic duct), which is responsive to a large number of carcinogens. The malignancies of the lung, in our opinion, were metastases from Zymbal gland tumours.

At that time (end of 1970) at the Institute of Oncology and Tumour Center of Bologna we planned a project of correlated investigations to further clarify the type and degree of the carcinogenic risk from VC.

The project was encouraged and supported by Montedison (Italy); the latter was joined by other major European companies, that is ICI (U.K.), Solvay (Belgium) and Rhone Progil (France).

The Head of the Health Services of Montedison supplied us with all the technical data dealing with occupational exposure. This enabled the approximation of the most important parameters of occupational conditions [illegible].

Our present knowledge on the carcinogenicity of VC is based upon experimental results and clinical-epidemiological data.

III. Experimental Data

1) Experiments performed at the Institute of Oncology and Tumour Center in Bologna, Italy

A) Planning, materials and methods.

Our project includes a series of experiments begun in sequence. They were designed to study the effects of VC administered through different routes (inhalation, ingestion, peritoneal and subcutaneous injection), at different concentrations, for varying periods of time (*continuous or intermittent treatment*), on animals of different species (rats, mice, hamsters), strains (Sprague Dawley and Wistar rats), sex and age (rabbits: new borns, embryos).

Program planning and preparation of suitable experimental conditions, particularly construction of an inhalatory exposure apparatus, took us until May, 1971. Experiments were started in July that same year.

The *chambers* of exposure are basically built of stainless steel and glass. They have been designed to deliver concentrations varying from 50,000 ppm to 1 ppm of VC and to simultaneously treat nearly 1,500 rodents. The VC concentrations in the chambers are controlled by gas chromatography.

VC is supplied by Montedison and each batch is analysed before use in Montedison Research Unit. The impurities and their maximum levels in the VC employed have been as follows:

H_2O	100 ppm
Acetic Aldehyd	5 ppm
Acetylene	2 ppm
Allene	5 ppm
Butane	8 ppm
1,3 Butadiene	10 ppm
Chloroprene	10 ppm
Diacetylene	4 ppm
Vinyl Acetylene	10 ppm
Propine	4 ppm
Methyl Chloride	100 ppm

Sprague Dawley rats are mostly employed [illegible] Wistar rats, Swiss mice and hamsters are also used. All the animals (except the hamsters) are kept [illegible] for a period of years and, regardless of their [illegible] animals [illegible] undergo autopsy. This gives us their extensive, critical pathological

The animals are weaned and classified by sex when 4-5 weeks old, at which time they are identified by *an ear punch*, and divided *into groups by litter distribution*. From weaning the animals are fed, ad libitum, an adequate commercial diet. During treatment, they are housed in groups of 10 in stainless steel wire cages with a solid bottom of the same material. After experimental treatment has ended the animals are kept in groups of 5 in similar cages with tops made of stainless steel wire. A shallow layer of white wood shavings serves as bedding. The animals are kept in a temperature controlled laboratory (20-24° C).

Different experiments (BT1-15) were started. Some of them were programmed at the beginning, while others began after results were obtained from the initial experiments. The experimental plan and current situation until September 10, 1974 are shown in table 1.

Experiment 1 studies the effect of the atmospheric exposure to 10,000, 6,000, 2,500, 500, 250, 50 ppm of VC, 4 hours daily, 5 days weekly, for 12 months. Two other groups of animals were added as controls: one untreated, as for all subsequent experiments, and one treated with vinyl acetate (VA) at 2,500 ppm, administered under the same conditions as VC. *This VA dosage appeared to be the maximum amount for chronic exposure.*

Experiment 2 investigates in a larger number of animals the effect of doses between 250 to 50 ppm, that is 200, 150 and 100, under the same conditions of experiment 1. This was started after it was clear that 250 ppm was still causing oncogenic effects.

Experiment 3 studies the effects of a shorter exposure period under the same conditions of experiment 1.

Experiment 4 studies the effects of the same type and degree of exposure as in experiment 1 on another species, that is mice. Treatment lasted 7 months because of the higher mortality rate and shorter life span of our mice as compared with rats.

Experiment 5 was undertaken as a pilot investigation to study the possible effects of atmospheric exposure during pregnancy on offspring.

Experiment 6 reproduces the same conditions of Professor Viola's experiments.

Experiment 7 studies the effects of the same exposure as in experiment 1 in another strain of rats, that is Wistar.

Experiment 8 studies the effects of the same exposure as in experiment 1 in a third species, that is hamsters, for the same period as in experiment 4.

Experiment 9 was planned, before tumours were observed at 50 ppm in experiment BT1, to further assess whether inhalation of VC has any effect at a dose level of 50 ppm.

Experiment 10 studies whether a high, but limited and/or intermittent atmospheric exposure has any effect.

Experiment 11 evaluates the effects of VC by ingestion.

Experiment 12 studies the third general route of exposure.

Experiment 13 assesses whether VC acts on tissues directly or through metabolites.

Experiment 14 has been included to study the responsiveness of newborns.

Experiment 15 was started as soon as we found 1 liver angiosarcoma, 1 extrahepatic angiosarcoma and 1 nephroblastoma, in three animals of the first experiment, exposed to 50 ppm of VC for 1 year, and surviving 135 weeks from the beginning of the treatment.

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B) *Results.*

The current st
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[illegible]rogrammed at the [illegible] experiments. The [illegible] shown in table 1.

[illegible], 6,000, 2,500, 500, [illegible]. Two other groups [illegible]equent experiments, [illegible]ted under the same [illegible] amount for chronic

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[illegible]etabolites,

[illegible]trahepatic angio[illegible] exposed to 50 [illegible] the treatment.

The animals are controlled weekly and weighed [illegible] two weeks during the treatment period, and monthly after treatment is completed. [illegible] detectable gross pathological [illegible] are recorded during control. All animals are kept under observation until spontaneous [illegible]. The animals, when moribund, are isolated, [illegible] [illegible] [illegible].

A complete autopsy is made [illegible] [illegible] animal [illegible] [illegible] [illegible] on Zymbal glands, interscapular [illegible] fat, [illegible] [illegible] [illegible], lungs, [illegible] spleen, stomach, different segments of the [illegible] [illegible] [illegible] [illegible] feet and any other organ with pathological [illegible].

All animals exposed to the highest dose [illegible] [illegible] [illegible] tumours are examined radiologically during treatment [illegible] [illegible] [illegible] examinations are made on animals bearing tumours [illegible] exposed to a [illegible] [illegible].

B) *Results*

The current results until September 10 of several experiments (BT1, [illegible] 2, 3, 7, 5, 4, 8), are shown in tables 2-9. For the latter experiments, data are not yet available.

Different types of tumours may be present in the same animals.

Zymbal gland carcinomas (in rats) may be bilateral. Histologically they frequently reproduce, to a certain extent, the structure of the normal Zymbal gland, but often they present squamous, glandular, solid, anaplastic and polymorphous patterns. Several of these different pictures may be observed in the same tumour. Metastases of these tumours are sometimes found in the lung.

Nephroblastomas (in rats) are often bilateral. Their histological picture reminds us of an embryonal kidney. They metastasize to the liver, spleen, lung and brain. Hyperplasia of nephroblastoma-like tissue is often observed in the kidney of treated rats, with or without nephroblastoma.

Angiosarcomas and angiomas (in rats, mice, hamsters) may be found at more than one site in the same animal. In the liver they are often polycentric. Hepatic and extrahepatic angiosarcomas are more or less differentiated. They are morphologically similar in rats, mice and hamsters. Angiosarcomas frequently metastasize and, as far as liver angiosarcomas are concerned, the lung is the most frequent site of distant metastases.

Blood vessels ectasias, endothelial hyperplasia, associated or not associated with cellular atypias, are often observed in the liver and in other organs and tissues in treated animals, with or without local or distant angiosarcomas or angiomas. Therefore, the effect of VC on blood vessels and endothelia should be considered systematic. Angioblastic proliferation, angiomas and angiosarcomas in the liver and other sites may or may not be associated with fibroplasia.

From the histological observations, the sequence of local changes in the genesis of malignant vascular tumours appears to be as follows: endothelial hyperplasia, angiomas, atypias of endothelial cells in hyperplastic [illegible] [illegible] and angiomas, angiosarcomas.

Fibro-angioblastic proliferation undergoing fibrosis is frequently [illegible] in the spleen of the treated rats and mice.

SPI-03208

TABLE I
PLAN OF THE EXPERIMENTS UP TO SEPTEMBER 10, 1974

No. of the experiments	TREATMENT			ANIMALS							Lenght of the experiments at the present date (weeks)
	Route	Doses of VC	Lenght	Species	Strain	Age (weeks)	N				
							♂	♀	Total	Per group	
BT1	Inhalation	10.000, 6.000, 2.500, 500, 250, 50 ppm. Untreated controls Treated controls: VA, 2.500 ppm.	4 hrs. daily, 5 days weekly, 52 weeks	Rat	Sprague-Dawley	13	1268	309	577	64-96	135 (Ended)
BT2	Inhalation	200, 150, 100 ppm. Untreated controls	4 hrs. daily 5 days weekly, 52 weeks	Rat	Sprague-Dawley	13	280	265	545	120-185	61
BT3	Inhalation	10.000, 6.000, 2.500 500, 250, 50 ppm. Untreated controls	4 hrs. daily, 5 days weekly, 17 weeks	Rat	Sprague-Dawley	21	262	288	550	60-190	86
BT4	Inhalation	10.000, 6.000, 2.500, 500, 250, 50 ppm. Untreated controls	4 hrs daily, 5 days weekly, 30 weeks	Mouse	Swiss	11	250	260	510	60-150	61
BT5	Transplacental	10.000, 6.000 ppm.	4 hrs. daily, 7 days (from 12 th to 18th day of pregnancy)	Rat	Sprague-Dawley	19 Breeders 12 days Embroys	110	36	146	30-54	95
BT6	Inhalation	30.000 ppm.	4 hrs. daily, 5 days weekly, 43 weeks	Rat	Sprague-Dawley	17	30	30	60	60	61
BT7	Inhalation	10.000, 6.000, 2.500, 500, 250, 50 ppm. Untreated controls	4 hrs. daily, 5 days weekly,	Rat	Wistar	11	–	220	220	30-40	61
BT8	Inhalation	10.000, 6.000, 2.500 500,250, 50 ppm. Untreated controls	4 hrs daily, 5 days weekly, 30 weeks	Hamster	Golden	11	–	268	268	32-70	48
BT9	Inhalation	50 ppm. Untreated controls	4 hrs. daily 5 days weekly, 52 weeks	Rat	Sprague-Dawley	11	200	200	400	100 (c) 300 (t)	33
BT10	Inha-	10.000, 6.000 ppm.	4 hrs.	Rat	Sprague-	11	420	420	840	120	33

	tal		from 12 th to 18th day of pregnancy)			days Embroys					
BT6	Inhalation	30.000 ppm.	4 hrs. daily, 5 days weekly, 43 weeks	Rat	Sprague-Dawley	17	30	30	60	60	61
BT7	Inhalation	10,000, 6,000, 2,500, 500, 250, 50 ppm. *Untreated controls*	4 hrs. daily, *5 days weekly,*	Rat	Wistar	11	–	220	220	30-40	61
	lation	500,250, 50 ppm. Untreated controls	daily, 5 days weekly, 30 weeks	ster							
BT9	Inhalation	50 ppm. Untreated controls	4 hrs. daily 5 days weekly, 52 weeks	Rat	Sprague-Dawley	11	200	200	400	100 (c) 300 (t)	33
BT10	Inhalation	10.000. 6,000 ppm. Untreated controls	4 hrs. daily, 5 days weekly, 5 weeks: 4 hrs. daily, 1 day, weekly, 25 weeks: 1 hr. daily, 4 days weekly, 25 weeks	Rat	Sprague-Dawley	11	420	420	840	120	33
BT11	Ingestion	50 mg., 16,65 mg., 3,33 mg./Kg. body weight in olive oil Controls: olive oil	5 times weekly 52 weeks	Rat	Sprague-Dawley	13	160	160	320	80	33
BT12	Endopetitoneal injection	4,25 mg. in 1.0 cc olive oil Controls: 1.0 cc olive oil	4, 3, 2, times by two months and once	Rat	Sprague-Dawley	13	150	150	300	60	33
BT13	Subcutaneous injection	4,25 mg. in 1,0 cc olive oil Controls: 1.0 cc olive oil	1 injection	Rat	Sprague-Dawley	21	80	70	150	75	33
BT14	Inhalation	10.000. 6.000 ppm.	4 hrs. daily, 5 days weekly, 5 weeks	Rat	Sprague-Dawley	1 day	45	44	89	43-46	28
BT15	Inhalation	25. 10. 5. 1 ppm. Untreated controls	4 hrs. daily 5 days weekly, 52 weeks	Rat	Sprague-Dawley	13	300	300	600	120	18

TABLE 2

EXPERIMENT BT1: RESULTS AFTER 135 WEEKS (END OF THE EXPERIMENT)

Groups and treatment	Animals (Sprague-Dawley rats)		ANIMALS WITH TUMOURS															
			Zymbal gland carcinomas (b)			Nephroblastomas (c)			Angiosarcomas				Subcutaneous angiomas	Skin carcinomas	Hepatomas	Brain neuroblastomas	Other types and/or sites (m)	Total (u)
									Liver (d)			Other sites (e)						
	Total	Corrected number (a)	No.	%	Average latency time (weeks)	No.	%	Average latency time (weeks)	No.	%	Average latency time (weeks)	No.	No.	No.	No.	No.	No.	No.
I) VA 2,500 ppm.	96	49	–	–	–	–	–	–										
II) VC 10,000 ppm.	69	61	16	26	50	5	8	59	9	15	64	3 (e)	4	3	1	7	7 (n)	38
III) VC 6,000 ppmm.	72	60	7	12	62	4	7	65	13	22	70	3 (f)	3	1	1	3	8 (o)	31
IV) VC 2,500 ppm.	74	59	2	3	33	6	10	74	13	22	78	3 (g)	3	1	2	5	4 (p)	33
V) VC 500 ppm.	67	59	4	7	79	4	7	83	7	12	81	2 (h)	1	1	3		4 (q)	22
VI) VC 250 ppm.	67	59	–	–	–	6	10	80	4	7	79	2 (i)		4			3 (r)	19
VII) VC 50 ppm.	64	59	–	–	–	1	2	135	1	2	135	1 (l)	1	1			9 (s)	30
VIII) No treatment	68	58	–	–	–	–	–	–									10 (t)	6
Total	577	464	29	–	–	26	–	–	47	–		14	12	11	7	15	45	185

a) Alive animals after 26 weeks, when the first tumour (a Zymbal gland carcinoma) was observed. The percentages are referred to the corrected number.
b) Metastases to lung.
c) Metastases to liver, lung, spleen and brain.
d) Metastases to lung.
e) 1 angiosarcoma of the lips; 1 angiosarcoma of the nose; 1 intrabdominal angiosarcoma (next to liver).
f) 1 angiosarcoma in subcutaneous fibrosing angioma; 1 ossifying parauricular angiosarcoma; 1 intrabdominal angiosarcoma (next to liver)
g) 2 intrabdominal angiosarcomas (1 next to spleen and 1 next to ovary); 1 ossifying angiosarcoma of neck.
h) 1 pulmonary angiosarcoma; 1 angiosarcoma of uterus.
i) 1 intrabdominal angiosarcoma (next to spleen); 1 intrathoracic ossifying angiosarcoma.
l) 1 intrabdominal diffused angiosarcoma.
m) Several cases of breast fibroadenomas, adrenal and pituitary tumours (generally adenomas) have not been considered, since their distribution in the different groups does not vary.
n) 2 Zymbal gland adenomas; 3 mammary carcinomas; 1 neurilemmoma; 1 ovarian cystoadenocarcinoma.
o) 4 Zymbal gland adenomas; 1 salivary gland adenocarcinoma; 2 hepatic and 1 perritoneal angiomas.
p) 1 Zymbal gland adenoma; 1 mammary carcinoma; 2 ependymomas.
q) 1 mammary carcinoma; 2 lymphomas; 1 pulmonary fibrosarcoma.
r) 1 Zymbal gland adenoma; 1 mammary carcinoma; 1 lymphoma.
s) 3 Zymbal gland adenomas; 2 mammary carcinomas; 1 subcutaneous angiopericitoma; 3 uterine adenocarcinomas (1 with sarcomatous component).
t) 1 invasive acanthoma of Zymbal gland; 1 subcutaneous fibrosarcoma; 2 peritoneal fibroangiomas; 2 uterine adenocarcinomas (1 with sarcomatous component); 1 uterine leiomyosarcoma; 1 ovarin fibrosarcoma; 1 pulmonary rhabdomyosarcoma; 1 lymphoma.
u) Several animal with 2 or more tumours.

q) 1 mammary carcinoma; 2 lymphomas; 1 pulmonary fibrosarcoma.
r) 1 Zymbal gland adenoma; 1 mammary carcinoma; 1 lymphoma.
s) 3 Zymbal gland adenomas; 2 mammary carcinomas; 1 subcutaneous angiopericitoma; 3 uterine adenocarcinomas (1 with sarcomatous component).
t) 1 invasive acanthoma of Zymbal gland; 1 subcutaneous fibrosarcoma; 2 peritoneal fibroangiomas; 2 uterine adenocarcinomas (1 with sarcomatous component); 1 uterine leiomyosarcoma; 1 ovarin fibrosarcoma; 1 pulmonary rhabdomyosarcoma; 1 lymphoma.
u) Several animal with 2 or more tumours.

TABLE 3
EXPERIMENT BT6: RESULTS AFTER 61 WEEKS

Groups and treatments	Animals (Sprague-Dawley rats)			Animals with tumours								
				Zymbal gland carcinomas			Nephroblastomas	Angiosarcomas		Other type and/or site	Total (d)
								Liver	Other sites		
	Total	Corrected number (a)	Survivors	No.	%	Average latency time (weeks)	No.	No.	No.	No.	No.
1) VC 30,000 ppm.	60	60	4	30	50	43	–	13	1 (b)	6 (c)	36

(a) Alive animals after 24 weeks, when the first tumour (a Zymbal gland carcinoma) was observed.
The percentages are referred to the corrected number.
(b) 1 lung angiosarcoma.
(c) 3 Zymbal gland adenomas, 1 hepatic angioma, 1 ovarian angioma, 1 forestomach papilloma.
(d) Several animals with 2 or more tumours.

SPI-03212

TABLE 4
EXPERIMENT BT2: RESULTS AFTER 61 WEEKS

Groups and treatments	Animals (Sprague-Dawley rats)		Animals with tumours					
	Total	Survivors	Zymbal gland carcinomas	Nephro-blastomas	Angiosarcomas		Other type and or site	Total
					Liver	Other sites		
			No.	No.	No.	No.	No.	No.
I) VC 200 ppm.	120	88	-	-	1	-	2 (a)	3
II) VC 150 ppm.	120	83	-	-	-	-	1 (b)	1
III) VC 100 ppm.	120	80	-	-	-	-	1 (c)	1
IV) No treatment	185	135	1	-	-	-	1 (d)	2
Total	545	386	1	-	1	-	5	7

a) 1 subcutaneous fibroangioma; 1 mammary carcinoma.
b) 1 nasal osteosarcoma.
c) 1 forestomach papilloma.
d) 1 Zymbal gland adenoma.

SPI-03213

TABLE 5
EXPERIMENT BT3: RESULTS AFTER 86 WEEKS (a)

Animals	Animals with tumours						

(b) 1 Zymbal gland adenoma.

TABLE 5
EXPERIMENT BT3: RESULTS AFTER 86 WEEKS (a)

Groups and treatment	Animals (Sprague-Dawley rats)		Animals with tumours						
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Brain neuroblastomas	Other type and or site (d)	Total (1)
					Liver	Other sites			
	Total	Survivors	No.	No.	No.	No.	No.	No.	No.
I) VC 10.000 ppm.	60	9	6 (16)	(5)	(9)	1 (b) (3)	6 (7)	3 (c) (15)	15 (40)
II) VC 6.000 ppm.	60	16	4 (7)	(4)	(11)	(3)	2 (3)	7 (d) (9)	10 (27)
III) VC 2.500 ppm.	60	33	1 (2)	2 (4)	(9)	(3)	2 (2)	(7)	5 (23)
IV) VC 500 ppm.	60	37	(2)	(2)	(3)	(2)		2 (g) (7)	2 (13)
V) VC 250 ppm.	60	15		1 (4)	(2)	(1)		2 (h) (5)	3 (9)
VI) VC 50 ppm.	60	18				1 (e)		(3)	1 (3)
VII) No treatment	190	128						3 (i) (3)	2 (2)
Total	550	256	11 (22)	3 (19)	(34)	2 (12)	10 (12)	17 (49)	38 (117)

(a) Between brackets are recorded the tumours found in experiment BT1 after 86 weeks.
(b) 1 subcutaneous angiosarcoma.
(c) 1 orbital angiosarcoma.
(d) Several cases of breast fibroadenomas, adrenal and pituitary tumours (generally adenomas) have not been considered, since their distribution in the different groups does not vary.
(e) 1 parauricular fibrosarcoma; 1 nasal papilloma, 1 renal adenoma.
(f) 1 Zymbal gland fibroanpioma, 2 skin carcinomas, 1 subcutaneous angioma, 1 mammary carcinoma, 1 forestomach papilloma, 1 cranial osteoma.
(g) 2 lymphomas.
(h) 1 Zymbal gland adenoma, 1 retrobulbar fibroma.
(i) 1 Zymbal gland adenoma, 2 lymphomas.
(l) Several animals with 2 or more tumours.

TABLE 6
EXPERIMENT BT7: RESULTS AFTER 61 WEEKS

Groups and treatment	Animals (Wistar rats)		Animals with tumours (a)						
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Brain neuroblastomas	Other types and or sites	Total
	Total	Survivors			Liver	Other sites			
			No.	No.	No.	No.	No.	No.	No.
I) VC 10,000 ppm.	30	8	– (7)	1 (3)	2 (3)	– (1)	1 (2)	2 (b) (7)	5 (18)
II) VC 6,000 ppm.	30	13	– (1)	– (1)	– (2)	– (1)	– (1)	– (1)	– (6)
III) VC 2,500 ppm.	30	8	– (1)	– (1)	– (1)	– (2)	–	– (1)	– (5)
IV) VC 500 ppm.	30	16	– (1)	–	1	–	–	–	1 (1)
V) VC 250 ppm.	30	16	–	–	–		–	1 (c)	1
VI) VC 50 ppm.	30	22	–	–	–	–	–	–	
VII) No treatment	40	34	–	–	–	–	–	–	
Total	220	117	– (10)	1 (5)	3 (6)	– (4)	1 (3)	3 (9)	7 (30)

a) Between brackets are recorded the tumours found in male Sprague-Dawley rats of the experiment BT1, after 61 weeks.
b) *2 Zymbal gland adenomas.*
c) 1 angioma of the caecum.

TABLE 7
EXPERIMENT BT5: RESULTS AFTER 95 WEEKS

c) Lymphoma of the caecum.

TABLE 7
EXPERIMENT BT5: RESULTS AFTER 95 WEEKS

Groups and treatment	Animals (Sprague-Dawley rats)		Animals with tumours					
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Other type and/or site	Total
	Total	Survivors			Liver	Other sites		
			No.	No.	No.	No.	No.	No.
I) VC 10.000 ppm. Breeders	30	8	-					
II) VC 6.000 ppm. Breeders	30	2	-	-	-	-		-
III) VC 10.000 ppm. Offsprings	54	31	1		-	1 (a)		2
IV) VC 6.000 ppm. Offsprings	32	15	-			1 (b)		1
Total	146	56	1	-	-	2		3

a) Subcutaneous angiosarcoma on a 24 weeks old male.
b) Subcutaneous angiosarcoma on a 22 weeks old female.

TABLE 8

EXPERIMENT BT4: RESULTS AFTER 61 WEEKS

Groups and treatment	Rats			Corrected number (a)			Survivors			Pulmonary tumours (b) No.	%	Average latency time (weeks)	Mammary carcinomas (c) No.	%	Average latency time (weeks)	Liver angiosarcomas No.	[illegible] No.	[illegible] No.	[illegible] No.	Total No.
I) VC 10,000 ppm.	30	30	60	22	28	50	–	–	–	35	70	36	13	47	31	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
II) VC 6,000 ppm.	30	30	60	26	28	54	–	–	–	38	70	38	8	28	33	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
III) VC 2,500 ppm.	30	30	60	23	30	53	–	–	–	30	57	43	9	30	35	11	[illegible]	[illegible]	[illegible]	[illegible]
IV) VC 500 ppm.	30	30	60	[illegible]	29	[illegible]	–	2	2	38	66	41	[illegible]	24	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
V) VC 250 ppm.	30	30	60	[illegible]	[illegible]	[illegible]	3	1	4	31	53	42	11	32	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]
VI) VC 50 ppm.	[illegible]	[illegible]	60	27	30	[illegible]	1	[illegible]	10	1	2	56	16	33	[illegible]		[illegible]		[illegible]	[illegible]
VII) No treatment	[illegible]	[illegible]	150	74	67	141	24	34	58	4	3	53						–	[illegible]	[illegible]
Total	[illegible]	250	510	230	241	471	28	46	74	177			[illegible]			[illegible]	[illegible]	[illegible]	14	[illegible]

a) Alive animals after 16 weeks, when the first tumour (a mammary carcinoma) was observed. The percentages are re[illegible]ed to the corrected number.
b) Adenomas, some of which undergoing malignant transformation.
c) In females.
d) 4 liver fibroangiomas; 3 subcutaneous angiomas; 1 heart fibroangioma; 1 ossifying interscapular angioma.
e) 4 liver angiomas; 2 liver fibroangiomas; 1 subcutaneous angiosarcoma; 1 renal fibroangioma; 1 thymic angioma.
f) 3 liver angiomas; 3 subcutaneous angiosarcomas; 1 subcutaneous angioma; 2 intrabdominal angiosarcomas; 2 renal [illegible]iosarcomas; 1 pulmonary angioma.
g) 1 liver angioma; 3 liver fibroangiomas; 2 subcutaneous angiosarcomas; 1 subcutaneous angioma; 1 subcutaneous fibro[illegible]ioma, 3 intrabdominal angiosarcomas; 1 intrabdominal fibroangioma; 1 angioma of the caecum; 1 renal angiosarcoma; 1 pu[illegible]onary fibroangioma; 1 testicular fibroangioma.
h) 5 liver angiomas; 5 liver fibroangiomas; 2 intrabdominal angiosarcomas; 1 pulmonary angioma; 1 scrotal angioma.
i) 2 liver angiomas; 2 liver fibroangiomas; 1 subcutaneous angiosarcoma; 2 subcutaneous angiomas, 1 subc[illegible]neous fibroangioma; 1 intrabdominal angioma; 1 intrathoracic fibroangioma; 1 angioma of the interscapular fat pad.
l) 2 squamous carcinomas, 1 invasive acanthoma.
m) 5 squamous carcinomas; 1 acanthoma.
n) 1 squamous carcinoma; 2 acanthomas.
o) 1 acanthoma.
p) 1 Zymbal gland adenoma; 1 forestomach papilloma.
q) 1 lymphoma; 1 subcutaneous leiomyosarcoma; 1 forestomach papilloma; 1 Harderian gland ad[illegible]noma.
r) 1 forestomach papilloma; 1 parotid gland mixed tumour.
s) 1 Zymbal gland adenoma.
t) 1 Zymbal gland adenoma; 1 lymphoma; 1 Leydig cell tumour.
u) 1 parotid gland adenocarcinoma.
v) 1 lymphoma.
z) Several cases with 2 or more tumours.

o) 1 squamous carcinoma, 2 acanthomas.
p) 1 acanthoma.
q) 1 Zymbal gland adenoma; 1 forestomach papilloma.
r) 1 lymphoma; 1 subcutaneous leiomyosarcoma; 1 forestomach papilloma; 1 Harderian gland adenoma.
s) 1 forestomach papilloma; 1 parotid gland mixed tumour.
t) 1 Zymbal gland adenoma.
u) 1 Zymbal gland adenoma; 1 lymphoma; 1 Leydig cell tumour.
v) 1 parotid gland adenocarcinoma.
z) 1 lymphoma.
z) Several cases with 2 or more tumours.

TABLE 9
EXPERIMENT BT8: RESULTS AFTER 48 WEEKS

Groups and treatment	Animals (Golden hamsters)		Animals with tumours						
	Total	Survivors	Liver angiosarcomas	Skin trichoepithelomas (a)	Melanomas	Lymphomas	Forestomach papillomas and acanthomas	Other type and or site	Total (d)
			No.	No.	No.	No.	No.	No.	
I) VC 10,000 ppm.	35	9	-	3	-	-	-	-	3
II) VC 6,000 ppm.	32	8	-	2	1	1	3	1 (b)	5
III) VC 2,500 ppm.	33	11	-	1	-	-	4	-	4
IV) VC 500 ppm	33	13	1	1	-	1	1	1 (c)	4
V) VC 250 ppm	32	8	-	1	-	-	-	-	1
VI) VC 50 ppm	33	11	-	3	1	1	-	-	5
VII) No treatment	70	35	-	2	-	-	-	-	2
Total	268	95	1	13	2	3	8	2	29

a) Several cases with acanthosis and some undergoing malignant transformation.
b) 1 hepatocarcinoma.
c) 1 subcutaneous angioma.
d) Several animals with 2 or more tumours.

Skin carcinomas in treated rats and mice appear frequently to arise from sebaceous glands of which they may reproduce to some extent morphological characteristics.

Hepatomas (in rats) are usually well differentiated. Metastases have been observed in the lung.

Brain neuroblastomas (in rats) are very similar to the human medulloblastomas. Since they may be quite small and therefore not easily detected macroscopically, we are now making serial sections of the brains of the treated rats to obtain more precise information on their real incidence.

Lung adenomas (in mice) are usually multicentric: their number is higher in animals treated with heavier doses. Many of these tumours appear to undergo early malignant transformation.

Mammary carcinomas (in mice and rats) are usually well differentiated adenocarcinomas with monomorphous or polymorphous arrangement. Mammary carcinomas of mice, may be solitary or multiple, and their most distinctive feature is the frequency with which they present areas of squamous metaplasia.

Several of the tumours produced by VC are quite rare or exceptional. To our knowledge liver angiosarcomas, nephroblastomas and neuroblastomas have never been described as spontaneously occurring in rats. Liver and extrahepatic angiosarcomas have been induced only in a few instances in experimental rodents — rats, hamsters and mice — (for references see MANTON and LEE-TUCK, 1974). As far as we know this is the first time in which nephroblastomas and neuroblastomas have been experimentally reproduced.

The BT11 experiment which deals with the effect of VC administered by inhalation (stomach tube) is progressing well, showing a good survival rate of the animals (table 10).

TABLE 10
EXPERIMENT BT11: RESULTS AFTER 33 WEEKS

Groups and treatment	Animals (Sprague-Dawley rats)		Animals with tumours					
			Zymbal gland carcinomas	Nephroblastomas	Angiosarcomas		Other type and/or site	Total
	Total	Survivors	No.	No.	Liver No.	Other sites No.	No.	No.
[illegible]	80	74						
[illegible]	80	76						
[illegible]	80	77						
[illegible]	80	72				-		-
[illegible]	320	299				-		

C) *Conclusions*

1) Un[illegible] animals spec[illegible] as preliminar[illegible]

2) The [illegible] When given [illegible] nephroblasto[illegible] carcinomas. I[illegible] in mice, lung[illegible] mary carcino[illegible] other sites [illegible] early eviden[illegible] stomach pap[illegible] observed in [illegible]

3) In [illegible] 50 ppm.

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5) A [illegible] exposed for [illegible] that the ne[illegible] concerned.

6) The [illegible] i.e. Sprague [illegible] the strain [illegible]

7) The [illegible] gland carcin[illegible] 7 days, app[illegible]

8) The[illegible] a new orie[illegible] exposed to [illegible]

9) No[illegible] should be p[illegible] compound [illegible] allow a cor[illegible]

D) *Implem[illegible]*

Our d[illegible] sponsoring [illegible] Before [illegible] inducing in [illegible]

C) *Conclusions.*

1) Under our experimental conditions, VC produced tumours in the three animals species studied: rats, mice and hamsters (data should still be considered as preliminary in hamsters).

2) The range of induced tumours varies exclusively from species to species. When given by inhalation VC produces in rats: Zymbal gland carcinomas, nephroblastomas, angiosarcomas and angiomas of the liver and other sites; liver carcinomas, hepatomas, brain neuroblastomas and possibly mammary carcinomas; in mice, lung adenomas (frequently undergoing malignant transformation), mammary carcinomas of a peculiar type, angiosarcomas and angiomas of the liver and other sites and skin epithelial tumours; in hamsters, liver angiosarcomas and, as early evidence seems to suggest, skin trichoepitheliomas, lymphomas and forestomach papillomas and acanthomas. Incidentally, liver angiosarcomas have been observed in all three animal species.

3) In the BT1 and BT4 experiments, VC shows a cancerogenic effect at 50 ppm.

4) From BT1 experiment (the only one completed) a dose-response relationship is clearly shown, as far as angiosarcomas and nephroblastomas are concerned, in the lower dose ranges: from 500 ppm to 50 ppm for angiosarcomas and from 250 ppm to 50 ppm for nephroblastomas.

5) A comparison of the results available at the present moment in rats exposed for 12 months and 4 months (BT1 and BT3 experiments) shows that the neoplastic response, as far as angiosarcomas and nephroblastomas are concerned, is affected by the length of exposure to VC.

6) The comparison of the results obtained in rats of two different strains, i.e. Sprague-Dawley (BT1) and Wistar (BT7), presently seems to suggest that the strain factor considerably affects the neoplastic response.

7) The onset of two subcutaneous angiosarcomas and of one Zymbal gland carcinoma in the offspring of breeders exposed during pregnancy for 7 days, appears to indicate a transplacental effect of VC.

8) The observation of a few cases of developing angiosarcomas suggests a new orientation in the pathogenetic interpretation of acroosteolysis in workers exposed to VC.

9) No tumours have been observed in rats exposed to VA. However it should be pointed out that, under our experimental conditions, exposure to the compound caused the death of most of the animals within a period that did not allow a correct carcinogenicity test.

D) *Implementation of data*

Our data have been periodically transmitted to the European Economic Community sponsoring the project and are available to all interested parties.

Before the end of 1972, a short time after it was known that VC was inducing in rats not only Zymbal gland tumours but also nephroblastomas and

Other type and organ site No	Total No

liver angiosarcoma - these results were given to the Manufacturing Chemists Association (MCA) in the U.S. This information promoted clinical investigations which in December 1973, for the first time, identified liver angiosarcoma in a worker of a U.S. factory (B.F. Goodrich in Louisville, Kentucky) producing VC-PVC, as an occupational tumour.

Our up-to-date results have been published (MAITONI, 1973, MAITONI and LEVEMBL, 1974, a, b, in press), and reported in 1974 in two hearings held by Occupational Safety and Health Administration (OSHA) of the U.S. Department of Labor, and in many symposia, conferences and meetings organized by the American Cancer Society, Italian Department of Labor, Italian Superior Institute of Health, New York Academy of Science, EEC, International Agency for Research on Cancer (IARC), UNESCO, Italian Institute of Social Medicine and Bologna Institute of Oncology, Italian Federation of the Unions of Chemical Workers (FULC), International Federation of Chemical and General Workers' Unions (ICF) and Medichem.

2) EXPERIMENTS PERFORMED BY OTHERS

A) *Prof. Viola experiments (Italy)*

At the meeting of a Working Group on VC held by the International Agency for Research on Cancer (IARC) in Lyon on June 24-25 1974, Prof. VIOLA revealed that in histologically reviewing his material of past experiments, he also detected in rats treated with VC, angiosarcomas of the liver, together with other malignancies of the skin, lung and intestine. Moreover he communicated to have induced skin acanthomas and lung adenocarcinomas in rabbits treated with VC by inhalation for 4 hours daily, 5 days weekly, for a period of at least 15 months.

B) *Industrial BIO-TEST Laboratories' Experiments*

These experiments were started in September, 1973. In May, 1974 at a Working Group on the Toxicity of VC-PVC held by the New York Academy of Sciences, preliminary results revealed that also in that laboratory liver angiosarcomas, and mammary and pulmonary tumours were observed in mice exposed to VC by inhalation.

IV) EPIDEMIOLOGICAL DATA

1) LIVER ANGIOSARCOMA

Since the first discovery of an occupational liver angiosarcoma caused by exposure to vinyl chloride, 30 cases of liver angiosarcomas to our knowledge have been observed among workers of VC-PVC industries in the U.S. and several European countries (table 11). Most of these tumours arose before 1973 (the

TABLE 11

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TABLE II

REPORTED CASES OF LIVER ANGIOSARCOMA IN WORKERS EXPOSED TO VINLY CHLORIDE OR POLYVINYL CHLORIDE

No.	Country	Case	Birth Date	1st VC or PVC exposure	Diagnosis of angiosarcoma	Age at the diagnosis	Years from 1st exposure to diagnosis	Total years of exposure	Date of death	Occupation
1	U.S.A.	01	10.17.23	12/09/48	03/03/73	49	22	16	03.03/73	Polymerization
2	U.S.A.	02	08/19/33	11/15.55	05/00/70	36	14	13	09.28/71	Polymerization
3	U.S.A.	03	05.25/15	11/28/45	12/19/73	58	28	28	12/19/73	Polymerization
4	U.S.A.	04	01/15/24	07/06.52	08/19/67	43	15	15	01.07/68	Polymerization
5	U.S.A.	05	01/25/12	06/19/44	04/09/64	52	20	18	04/09/64	Polymerization
6	U.S.A.	06	00.00/29	01/17/62	02/00/74	45	12	12	living (09/00/74)	Polymerization
7	U.S.A.	07	05/03/22	08/00/44	00/00/68	45	24	18	03/23/68	Polymerization
8	U.S.A.	08	05/06/20	10/07/46	08/00/61	41	15	15	08.29/61	Polymerization
9	U.S.A.	09	00/00/31	05/28/45	03/01/74	43	29	17	living (09/00/74)	Polymerization
10	U.S.A.	10	08 16.13	06/12.51	05/00.68	55	17	17	05.10/68	Polymerization
11	U.S.A.	11	05 27.09	10/14/46	03.00.70	61	23	23	03 16/70	Polymerization
12	U.S.A.	12	11/17 18	09/13/49	05 02/69	50	20	15	05 02/69	Polymerization
13	U.S.A.	13	12 01.21	08.19/44	05 00/74	53	30	30	07 04/74	Polymerization
14	U.S.A.	14	00 00 13	08 18 38	06 00 73	60	36	00	07.03/73	PVC plastic insulation of electrical wire
15	U.S.A.	15	00 00 25	00.00 00	07 00 72	47	00	10	02 15/73	Accountant at several fabrication plants
16	U.S.A.	16	11 04 22	05.08.50	00 00 69	41	17	04 1.2	03 27/69	Polymerization
17	W. Germany	01	07 26 31	10.14 57	00 00 71	40	14	14	15 14 71	Polymerization
18	W. Germany	02	06 04 30	10.01 57	00 00 69	39	11	11	01 25 69	Polymerization
19	W. Germany	03	00 00 00	00 00 00	00 00 74	43	—	14	no DD 00	Loading personnel cars with VC propellant
20	Great Britain	01	00 00 01	00 00 46	12 00 72	71	26	20	12 00/72	Polymerization
21	Great Britain	02	09 08.14	00.00.46	02 00 70	55	24	11	12 00 70	Reacting VC and mixture onto fabric bases
22	Norway	01	12 33 15	03.00.50	11 00 71	56	15	15	01 04 72	Polymerization
23	Sweden	01	06 33.27	08/14 51	02 00 76	43	19	18	10 20.76	Polymerization
24	Sweden	02	11/27 11	00/00.45	05 15 72	61	27	23	08 16/72	Monomer production
25	Russia	01	00 00 00	00.00.00	00 00 76	00	00	00	no DD 00	—
26	Czechoslovakia	01	00 00 00	00/00.00	00 00 00	00	00	00	no DD 00	—
27	Czechoslovakia	02	00 00 00	00/00/00	00 00 00	00	00	00	no DD 00	—
28	Italy	01	06 15 34	00.00.65	04 19 71	36	06	03	04 16.71	Production of plastic sacks
29	Italy	02	11 11 39	00/00 57	12 13 73	43	15	06	12.00.73	Polymerization
30	France	01	00 00 23	00 00 00	00 00 00	47	19	19	00 00/77	Polymerization

... case dates from 1961), but in absence of experimental data, they ... not linked to the occupational exposure, and as a matter of fact, they often ... not properly diagnosed. The majority of cases did not occurr until 15 or ... year after the first exposure to vinyl chloride. This latency period is ... to that observed in other occupational tumours.

VI) Other Malignancies.

An excess mortality for cancers of the respiratory tract, blood forming tissues and brain has been observed among U.S. workers of VC polymerization facilities (J.K. Wagoner, Statement before the Subcommittee on the Environment of the U.S. Senate Commerce Committee, August 21, 1974) (See table 12).

The results of Dr. Wagoner's study are consistent with the mortality study undertaken at Mt. Sinai School of Medicine by Dr. I. Selikoff.

TABLE 12 (From Dr. Wagoner)
EXPECTED AND OBSERVED DEATHS FROM VARIOUS CANCER CAUSES AMONG SELECTED EMPLOYEES OF TWO (U.S.) VC-PVC POLYMERIZATION FACILITIES (1950-1973)

Years since first exposure	Person years at risk	All cancer		Liver cancer		Brain cancer		Respiratory system cancer		Lymphoma and leukemia		Stomach cancer	
		EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.
10-14		4.93	6	12	0	22	0	1.50	3	.61	1	2.46	3
15-19		6.36	11	.17	3 (1)	25	1	2.08	3	.67	2	3.13	3
20-24		5.81	12	.15	3 (1)	20	2 (1)	1.99	4	.55	1	3.92	3
25		2.72	2	.07	0	.06	1	.92	0	.24	0	1.43	1
Total	9,931	19.74	31 (1)	.51	6 (2)	.73	4 (1)	6.49	10	2.07	4	9.94	7
Standard Mortality Ratio													
Ratio		157		1.176		549		154		193		70	

(1) Significant at P = 0.05
(2) Significant at P = 0.01

When controls were made on employees of VC-PVC Italian factories (1970 and 1973) by sputum cytologic examinations, we noticed a high incidence of changes in the respiratory epithelium. Such changes consist of squamous metaplasia and typical adenomatous proliferation of varying degrees (Cytological classes II and II+), and more or less pronounced squamous dysplasia and atypical adenomatous proliferation (Classes II++ and III) (tables 13-15).

There are now to our knowledge various epidemiological investigations being undertaken in many parts of the world, such as the U.S., U.K., Italy, France, Germany, etc.

V) Measure of Prevention.

The only real effective preventive measure in occupational and environmental carcinogeneses is to avoid exposure to potential carcinogenic agents. That is the primary prevention.

EARLY RESULTS OF ...
OF VC-PVC MONTE...

Classes	
I	
I +	
II	
II + and II ++	
III	
Total	

EARLY EVALUATI...
FERATION AND OF ...
FACTORIES IN TERN...

Lenght of exposure (years)	Exposed workers N.
1- 5	5
6-10	15
11-15	5

RESULTS OF SP...
WORKERS O...

Classes	
I	
I +	
II	
II +	
II + +	
III	
Insufficient material	
Total	

SPI-03223

-imental data, they of fact, they often occurr until 15 or latency period is

...ct, blood forming VC polymerization e on the Environ- 74) (See table 12). with the mortality SELIKOFF.

...ED EMPLOYEES

...lymphoma and leukemia		Residual cancer	
EXP	OBS	EXP	OBS
61	1	2.46	2
67	2	3.13	2
33	1	2.93	2
34	0	1.43	1
5.07	4	9.04	7
143		70	

... Italian factories ...d a high incidence ...nsist of squamous ...ng degrees (Cyto- ...quamous dysplasia ...11) (tables 13-15).

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TABLE 13

EARLY RESULTS OF SPUTUM CYTOLOGICAL EXAMINATIONS AMONG WORKERS OF VC-PVC MONTEDISON FACTORIES IN TERNI AND BRINDISI (ITALY) (1974)

Classes	Exposed workers	Non exposed (controls)
I		
I +	7	4
II	3	10
II + and II + +	12	3
III	3	1
Total	25	18

TABLE 14

EARLY EVALUATION OF THE INCIDENCE OF SQUAMOUS METAPLASIA, ADENOMATOUS PROLIFERATION AND OF SQUAMOUS DYSPLASIA AMONG EXPOSED WORKERS OF VC-PVC MONTEDISON FACTORIES IN TERNI AND BRINDISI (ITALY) [illegible]

Length of exposure (years)	Exposed workers N	Squamous metaplasia N	%	[illegible] N	%	Adenomatous proliferation N	%	Squamous dysplasia N	%
1-5	8	4	50					1	[illegible]
6-10	15	11	73	[illegible]		[illegible]	[illegible]	1	[illegible]
11-15	5	2	40	2	[illegible]	1	[illegible]	1	[illegible]

TABLE 15

RESULTS OF SPUTUM AND URINE CYTOLOGICAL EXAMINATIONS AMONG WORKERS OF VC-PVC ANIC FACTORY IN RAVENNA (ITALY) (1973).

Classes	Exposed workers Sputum	Urine
I	30	33
I +	27	28
II	83	64
II +	12	4
II + +	2	
III	7	1
Insufficient material	63	94
Total	224	224

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In case of VC, a world-wide MAC standard has not yet been established. However in the U.S., OSHA has decided to reduce allowable VC air-levels to less than 1 ppm — the so-called non-detectable level — during the 8 hour working day, with a 5 ppm ceiling for a maximum 15 minutes daily. This decision followed the observation that the exposure to 50 ppm of VC in air was still causing tumours (including angiosarcomas) in rats and mice.

The MAC standard level, the MAC time period, the types of monitoring, and the control of the monitoring are major topics of discussion today among interested parties, i.e. unions, industries and governments.

Medical controls may achieve very *little*. In fact, changes produced by exposure to carcinogenic agents are irreversible, experimental and epidemiological evidence indicated that VC may produce tumours at different sites; occupational tumours — as is true in the case of occupational angiosarcoma — are usually multicentric, and finally, as regards to liver angiosarcoma, we have neither clinical nor laboratory methods for detecting its precursors, apart from laparoscopy and liver biopsy.

VC: the Lesson

It is increasingly demonstrated and accepted that the high and rising prevalence of tumours, especially in particular sites (lung, urinary tract, hemopoietic tissues), is due to the extensive and growing *diffusion* of oncogenic agents in the occupational and general environment. Furthermore it is basically assumed that 80 to 90% of tumours are related to causes which are, or may be identified as being, part of the human environment. Recognition of these causes and protection from their effects could significantly reduce tumour morbidity and mortality.

This increasing diffusion of environmental oncogenic agents is a phenomenon particularly correlated to the growth of industrialization, and especially to those industries producing and manufacturing new synthetic compounds. In modern days, *from 100,000 to 200,000* new chemical compounds are produced annually, yet we know very little about their effect on man.

Most of the oncogenic occupational and environmental agents have been identified when the incidence of a particular type of tumour in an exposed population has been outstanding and/or on the basis of retrospective epidemiological studies.

Progress in these areas of oncology should facilitate systematic experimental oncogenicity testing of agents which are, or will be, released in the occupational and general environment. These testing should be carried out before the exposure of humans to potentially oncogenic agents takes place (since the specific effect on tissues produced by carcinogens are irreversible) and before economical, political and social interests are well established (since then they can not easily be changed).

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Mouse	+	+
Hamster	+	+
Man	+	+

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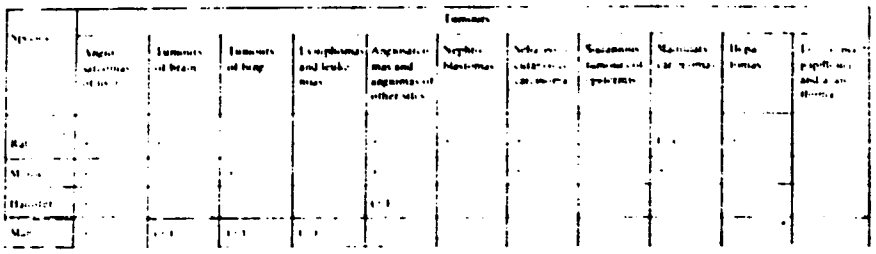
Carcinogenicity experimental bioassays are surprisingly far below the need. This is due to several factors: past scepticism concerning the validity of testing, vast amount of agents to be tested, complications in performing elaborate testings, lack of experimental units and confusion of responsibilities.

We believe that a permanent intergovernmental commission, together with representatives from interested parties, should: 1) plan the program and priorities, 2) promote at an international level the adequate and interactive network of experimental units, and 3) critically evaluate the results and decide proper preventive measures.

VC carcinogenesis has shown the value of experimental bioassays in predicting oncogenic risks. In one or more of the three animal species we tested, VC has caused until now all types of tumours which, based on available epidemiological data, are being correlated to occupational exposure, i.e. liver angiosarcomas, brain and lung tumours, and lymphomas and leukemias (table 16).

TABLE 16

TUMOURS PRESENTLY CORRELATED TO VC EXPOSURE BY INHALATION ON EXPERIMENTAL ANIMALS AND MAN



Furthermore, experimental bioassays on VC carcinogenicity have provided a basis for assessing new MAC standards.

It is our hope that the story of VC carcinogenicity may serve as a convincing example to urgently promote experimental bioassays for all new industrial compounds and for those compounds which are already produced and widespread but whose effects we still ignore.

VIII. Our Ongoing Projects

I) VC carcinogenicity bioassays:

A) A complete statistical analysis of available data is now in progress which will be published shortly.

B) We are awaiting the completion of experiments currently in hand, particularly BT10 and BT15, which may supply us with information on the effects of heavy exposure for short and/or intermittent periods of time and prolonged exposure below 50 ppm.

C.) Studies are now going on to acquire knowledge of the morphologic and metabolic precursors of tumours produced by VC, with particular reference to liver angiosarcomas in different species.

2) We have started new experiments for cancerogenicity bioassays of other monomers heavily produced by plastic industries.

SUMMARY

Available experimental and epidemiological data on carcinogenesis by VC are reviewed.

The experimental bioassays on the oncogenic potentialities of VC, performed at the Bologna Institute of Oncology and Tumour Center, are described in detail.

Based on these experiments, when given by inhalation, VC produces: in rats, Zymbal gland carcinomas, nephroblastomas, angiosarcomas and angiomas of the liver and other sites, skin carcinomas, hepatomas, brain neuroblastomas, and possibly mammary carcinomas; in mice, lung adenomas (frequently undergoing malignant transformation), mammary carcinomas of a peculiar type, angiosarcomas and angiomas of the liver and of other sites, and skin epithelial tumours; in hamsters, liver angiosarcomas and, as early evidence seems to suggest, skin trichoepitheliomas, lymphomas, and forestomach papillomas and acanthomas.

This early results of the Bologna experiments promoted clinical and epidemiological investigations which led to the discovery of occupational liver angiosarcomas among workers of VC/PVC industries.

The story of VC carcinogenesis suggests the need of experimental carcinogenicity bioassays for all industrial compounds before they are produced and widespread.

RIASSUNTO

MALTONI C., LEFEMINE G., CHIECO P., CARRETTI DONATY — *Cancerogenesi da cloruro di vinile: risultati attuali e prospettive*

Sono stati passati in rassegna i dati sperimentali ed epidemiologici sulla cancerogenesi da CV.

I saggi sperimentali sulle potenzialità oncogene del CV, condotti presso l'Istituto di Oncologia e Centro Tumori di Bologna, sono stati riferiti in dettaglio.

Sulla base di questi esperimenti, il CV, quando somministrato per inalazione, produce: nei ratti, carcinomi delle ghiandole di Zymbal, nefroblastomi, angiosarcomi ed angiomi del fegato e di altri distretti anatomici, carcinomi della cute, epatomi, neuroblastomi encefalici, e probabilmente carcinomi mammari, nei topi, adenomi del polmone (in molti casi in trasformazione maligna), carcinomi mammari di tipo particolare, angiosarcomi e angiomi del fegato e di altri distretti anatomici, e tumori epiteliali della cute; nei criceti, angiosarcomi del fegato e, come dati preliminari sembrano indicare, tricoepiteliomi cutanei, linfomi, e papillomi e acantomi del prestomaco.

I primi risultati degli esperimenti di Bologna hanno promosso le indagini cliniche ed epidemiologiche che hanno portato alla scoperta di angiosarcomi epatici professionali tra lavoratori di industrie di CV-PCV.

La storia della cancerogenesi da CV sta ad indicare la necessità di saggi sperimentali di cancerogenicità per tutti i composti industriali, prima che essi siano prodotti e diffusi a larga scala.

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RESOCONT

Ricerche
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ENVIRONMENTAL RESEARCH 7, 387–405 (1974)

Carcinogenicity Bioassays of Vinyl Chloride

I. Research Plan and Early Results[1]

CESARE MALTONI AND GIUSEPPE LEFEMINE

Istituto di Oncologia "F. Addarii" and Centro Tumori, Bologna, Italy

Received March 11, 1974

These investigations are concerned with determining the oncogenic potentialities of vinyl chloride (VC) in experimental animals. The effect of this compound is being studied in relation to various experimental factors, such as route of administration, dose level, length of treatment, species, strain, and age of the animals.

Preliminary results are presented. When given by inhalation, VC induces in rats, Zymbal gland carcinomas, nephroblastomas, and angiosarcomas of the liver and of other anatomical sites; and in mice, pulmonary adenomas, mammary carcinomas and liver angiosarcomas.

A direct relationship has been found between dose and length of treatment and neoplastic response.

Vinyl chloride (VC) ($CH_2 = CHCl$) is a chemical compound of increasing industrial importance. It is used in the production of polyvinyl chloride resin, as a copolymer in Saran and other plastics, as a chemical intermediate, as a solvent, and as a propellent; in the past, for a short period, it was also used as anaesthetic. It is at present produced at a rate of near 12 million tons per year.

The following population groups may be exposed to VC: (1) workers engaged in the production of polyvinyl chloride (PVC) and of VC, and in the use of VC for other industrial purposes; (2) workers manufacturing PVC; (3) residents about factories producing PVC and VC; and (4) to a much lesser extent, people undergoing contact with resins made with VC, with materials containing these resins or consumer products containing VC, as some propellant sprays.

The present research includes a series of correlated and integrated experiments dealing with carcinogenicity bioassays of this monomer. This research followed the preliminary results of the pioneering work of P. L. Viola, presented at the X International Cancer Congress (Houston, 1970) and published in 1971 (Viola *et al.*, 1971).

Viola's results indicated that exposure to 30,000 ppm of VC, 4 hours a day, 5 times a week for 10 months, produced tumours in rats, and that these occurred in skin, lungs and bones.

Immediately after the report in Houston, we communicated with Professor Viola, who kindly put at our disposal the manuscript of his extensive report, then in press, and several slides of the tumours.

On the basis of his results and material, we reached the following conclusions.

[1] This project has been supported by the European Cooperative Group (ECG) which includes Montedison (Italy), ICI (U. K.), Solvay (Belgium), Rhone-Progil (France).

SPI-03228

(1) Under Professor Viola's experimental conditions, VC definitely showed a carcinogenic effect on rats;
(2) 30,000 ppm of VC was an extremely high level of exposure;
(3) The tumours described as cutaneous arose from Zymbal glands, which in rats are responsive to a large spectrum of carcinogens: e.g., 2-acetyl-amino-fluorene (Wilson *et al.*, 1941), benzidine (Spitz *et al.*, 1950), 3,2-dimethyl-4-aminobiphenyl (Walpole *et al.*, 1952), 4-aminostilbenes (Haddow *et al.*, 1948), 9,10-dimethyl-1,2-benzanthracene (Geyer *et al.*, 1953), 3-methoxy-4-aminoazobenzene (Miller and Miller, 1961), urethan (Tannenbaum *et al.*, 1962), and others.
(4) The pulmonary tumours were most likely metastases from Zymbal gland carcinomas. These lesions were in fact morphologically similar to the Zymbal gland carcinomas, and all were observed in animals bearing Zymbal gland tumours.

It appeared useful to further investigate the nature and extent of the carcinogenic effect of VC, with particular emphasis on experimental factors related to exposure, such as route, concentration, duration, continuity or intermittence, etc., which could give more direct information concerning the risk of occupational and environmental exposure, and other factors related to the animals studied, such as species, strain, sex, and age. For these reasons, we were contacted by Professor Bartalini, Director of the Health Services of Montedison who offered the support of his Company for an experimental study of the biological effect of VC. Montedison was soon joined by ICI, Solvay and Rhone-Progil.

MATERIALS AND METHODS

The construction of an apparatus for programmed and controlled exposure took several months. The chambers were designed to deliver concentrations varying from 30,000 ppm to 50 ppm and to simultaneously treat nearly 1200 experimental rodents. Basically, it is built of stainless steel and glass. The control of VC concentrations is by gas chromatography.

VC has been supplied by Montedison. Each batch has been analyzed in the chemical research laboratory of Montedison before use to assess its degree of purity (99.99%).

The experiments largely utilized Sprague–Dawley rats. Wistar rats, Swiss mice, and hamsters have also been used. All the animals, except the hamsters, have been bred in our Institute for years and, whatever their use, are all examined at death by complete autopsy, giving us extensive information concerning their current pathology.

The animals have been weaned and classified by sex when 4–5 weeks old, at which time they have been numbered by ear punch, and divided into groups by litter distribution. From weaning, the animals have been fed, *ad libitum*, an adequate commercial diet. During the period of treatment, the animals are housed in groups of 10 in stainless steel wire cages with a solid bottom of the same metal. After the period of experimental treatment, the animals are kept in groups of 5 in makrolon cages, with tops made of stainless steel wire. A

shallow layer of white wood shavings served as bedding. The animals were kept in a temperature controlled laboratory at 19–20°C.

Fourteen experiments have been started in sequence. Some were programmed at the onset. The need for others appeared on the basis of initial experimental results. The plan of the experiments is shown in Table 1.

Experiment 1

Experiment 1 was to investigate the effect of the atmospheric exposure to 10,000, 6000, 2500, 500, 250, and 50 ppm of VC, 4 hours daily, 5 days weekly, for 12 months. Two groups of animals were added as controls: one untreated, as with all the following experiments, and one treated with vinyl acetate (VA) at 2500 ppm, under the same conditions. This dose of VA appeared to be the maximum possible dose for a chronic exposure.

Experiment 2

Experiment 2 was performed to obtain data in a larger number of animals on the effect of doses between 250 and 50 ppm, i.e., 200, 150, and 100 ppm, under the same conditions as in Expt 1. It was begun after it became clear that 250 ppm still showed oncogenic effects.

Experiment 3

This was planned to study the effects of a shorter period of exposure, keeping constant the other conditions of Expt 1.

Experiment 4

Experiment 4 studies the effect of the same type of exposure as in Expt 1, in another species, i.e., mice. The treatment lasted 7 months in view of the higher mortality, and the shorter life span of the mice of our line.

Experiment 5

This was undertaken as a pilot investigation on the possible effects of the atmospheric exposure during pregnancy, on offspring.

Experiment 6

Experiment 6 reproduced the conditions of Viola's experiments.

Experiment 7

This experiment was designed to study the effects of the same type of exposure as in Expt 1 in another strain of rats, i.e., Wistar.

Experiment 8

Experiment 8 studies the effects of the same exposure as in Expt 1 in a third species, i.e., hamsters, for the same period as in Expt 4.

Experiment 9

This was planned to further assess whether or not inhalation of VC has any effect at a dose level of 50 ppm in experimental animals.

TABLE 1

PLAN OF THE EXPERIMENTS TO MARCH 11, 1974

No. of experiment	Treatment			Animals							Length of experiments (weeks)
							No.				
	Route	Doses of VC	Length	Species	Strain	Age (weeks)	♀	♂	Total	Per group	
BT1	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls Treated controls: VA 2500 ppm	4 hours daily, 5 days weekly, 52 weeks	Rat	Sprague-Dawley	13	268	309	577	64-98	131
BT2	Inhalation	200, 150, 100 ppm Untreated controls	4 hours daily, 5 days weekly, 52 weeks	Rat	Sprague-Dawley	13	280	265	545	120-185	35
BT3	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 17 weeks	Rat	Sprague-Dawley	21	202	288	550	60-190	60
BT4	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 30 weeks	Mouse	Swiss	11	250	260	510	60-150	35
BT5	Transplacental	10,000, 6000 ppm	4 hours daily, 7 days of pregnancy	Rat	Sprague-Dawley	10 Breeders 12 days Embryos	110	30	140	30-54	69
BT6	Inhalation	30,000 ppm	4 hours daily, 5 days weekly, 52 weeks	Rat	Sprague-Dawley	17	30	30	60	60	35
BT7	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 52 weeks	Rat	Wistar	11	--	220	220	30-40	35
BT8	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 30 weeks	Hamster	Golden	11	--	268	268	32-70	22
BT9	Inhalation	50 ppm (f)	4 hours daily, 5 days	Rat	Sprague-	11	200	200	400	100 (e)	7

BT7	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 52 weeks	Rat	Wistar	11		[illegible]	220	220	[illegible]
BT8	Inhalation	10,000, 6000, 2500, 500, 250, 50 ppm Untreated controls	4 hours daily, 5 days weekly, 30 weeks	Hamster	Golden	11	—	268	268	32-70	22
BT9	Inhalation	50 ppm (*t*) Untreated controls (*c*)	4 hours daily, 5 days weekly, 52 weeks	Rat	Sprague-Dawley	11	200	200	400	100 (*c*) 300 (*t*)	7
BT10	Inhalation	10,000, 6000 ppm Untreated controls	4 hours daily, 5 days weekly, 5 weeks: 4 hours daily, 1 day weekly, 25 weeks; 1 hour daily, 4 days weekly, 25 weeks	Rat	Sprague-Dawley	11	420	420	840	120	7
BT11	Ingestion	16,6 mg; 3,32 mg; 50 mg kg body weight in olive oil Controls: olive oil	5 times weekly, 52 weeks	Rat	Sprague-Dawley	13	160	160	320	80	7
BT12	Endoperitoneal injection	4,25 mg in 1,0 cc olive oil Controls: 1,0 cc olive oil	4, 3, 2, times by two months, and once	Rat	Sprague-Dawley	13	150	150	300	60	7
BT13	Subcutaneous injection	4,25 mg. in 1,0 cc olive oil Controls: 1,0 cc olive oil	1 injection	Rat	Sprague-Dawley	21	80	70	150	75	7
BT14	Inhalation	10,000, 6000 ppm	4 hours daily, 5 days weekly, 5 weeks	Rat	Sprague Dawley	1 day	45	41	82	43-46	2

Experiment 10

Experiment 10 studies the effect of a high, but short and/intermittent atmospheric exposure.

Experiment 11

This was performed to evaluate possible effects of VC by ingestion. Doses have been chosen on the basis of early data on the release of VC from food containers made of PVC, and following suggestions of EEC.

Experiment 12

This experiment studies a third general route of exposure namely the peritoneal cavity.

Experiment 13

Experiment 13 was started to assess whether the VC acts on tissues directly or through metabolites.

Experiment 14

This experiment has been included to study the responsiveness of newborn animals.

The animals are examined weekly, and weighed every 2 weeks during the period of treatment, and monthly after the treatment is completed. All detectable gross pathological changes are recorded during examination. All animals are kept under observation until spontaneous death. Moribund animals are isolated, in order to avoid cannibalism.

A complete autopsy is made of every animal. Histological specimens include Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bones of the legs and feet, and any other organ with pathological lesions. The tissues are fixed in alcohol, processed, sectioned, and routinely stained by hematoxylin and eosin and with Van Gieson's method; in selected cases, other staining techniques have also been used.

All animals exposed to the highest doses (30,000 and 10,000 ppm) with or without tumours, are examined radiologically, during treatment and/or at death. Moreover, radiological examinations are made of all animals bearing tumours, even if exposed to the lower doses.

RESULTS

Preliminary results and other current data of Expts BT1, BT3, and BT4 are given in Tables 2–4.

Special aspects of Expt BT5 and BT6 are discussed in the text. Insofar as the other experiments are concerned, results are awaited.

In Table 2 are listed the observations on the oncogenic effect of graded concentrations of VC within a range of 50–10,000 ppm. It is evident that the overall occurrence of tumours decreases with decreasing concentrations from 39% tumour

Groups at treatmen

I
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TABLE 2
EXPERIMENT BT1: RESULTS AFTER 131 WEEKS

Groups and treatment	Animals (Sprague Dawley rats)		Animals with tumours					
			Zymbal glands carcinomas[a]	Nephroblastomas[b]	Angiosarcomas		Other type and/or site	Total
	Total	Survivors			Liver	Other sites		
			No.	No.	No.	No.	No.	No.[m]
I VA 2500 ppm	96	—	—	—	—	—	—	—
II VC 10,000 ppm	69	—	13	3	6	—	5[k]	27
III VC 6000 ppm	72	—	5	3	11	1[d]	1[e]	21
IV VC 2500 ppm	74	—	2	6	9	3[c]	1[f]	21
V VC 500 ppm	67	—	3	3	7	2[f]	1[g]	16
VI VC 250 ppm	67	1	—	5	2	2[g]	2[j]	11
VII VC 50 ppm	64	3	—	—	—	—	—	—
VIII No treatment	68	1	—	—	—	—	—	—
Total	577	5	23	20	35	8	10	96

[a] Metastases to lung.
[b] Metastases to liver and/or to lung and spleen.
[c] Metastases to lung.
[d] Angiosarcoma in subcutaneous fibrosing angioma.
[e] Two intra-abdominal angiosarcomas (1 contiguous to spleen and 1 to ovary); 1 ossifying angiosarcoma of neck.
[f] One pulmonary angiosarcoma; 1 angiosarcoma of uterus.
[g] One intra-abdominal angiosarcoma (near to spleen); 1 intrathoracic ossifying angiosarcoma.
[h] Two Zymbal gland adenomas; 1 neurilemmoma of the ear; 1 mammary carcinoma; 1 cystoadenocarcinoma of ovary.
[i] Sebaceous gland carcinoma of skin.
[j] Zymbal gland adenoma.
[k] *Minimal deviation hepatoma.*
[l] One Zymbal gland adenoma; 1 salivary gland carcinoma.
[m] Total No. of tumours.

incidence at 10,000 ppm, 29% at 6000 and 2500 ppm to 24% and 16% at 500 and 250 ppm, respectively. Fifty (50) ppm did not cause tumour development in the limited number of animals so exposed, and no neoplasms were found in untreated controls and in rats exposed to VA 2500 ppm. Zymbal gland carcinomas, nephroblastomas and angiosarcomas of the liver and other sites were the prevailing tumours. The development of the latter 2 neoplasms was not always dependent on the concentration of the toxic agent, but to a higher degree on the duration of exposure. If exposure time was reduced from 52 weeks (Table 2) to 17 weeks (Table 3) the number of tumours after 60 weeks is markedly smaller: their per-

TABLE 3
EXPERIMENT BT3: RESULTS AFTER 60 WEEKS[a]

Groups and treatment	Animals (Sprague-Dawley rats) Total	Survivors	Animals with tumours: Zymbal glands carcinomas No.	Nephroblastomas No.	Angiosarcomas Liver No.	Angiosarcomas Other sites No.	Other type and or site No.	Total No.[b]
I VC 10,000 ppm	60	36	3 (11)	— (1)	— (1)	—	— (4)	3 (17)
II VC 6000 ppm	60	48	1 (3)	— (1)	— (3)	— (1)	—	1 (8)
III VC 2500 ppm	60	54	— (2)	— (1)	— (1)	—	— (1)	— (5)
IV VC 500 ppm	60	56	— (1)	—	— (1)	—	— (1)	— (3)
V VC 250 ppm	60	44	—	—	— (1)	—	—	— (1)
VI VC 50 ppm	60	50	—	—	—	—	—	—
VII No treatment	190	183	—	—	—	—	—	—
Total	550	471	4 (17)	— (3)	— (7)	— (1)	— (6)	4 (34)

[a] Tumours found in experiment BT1 after 60 weeks are recorded in parenthesis.
[b] Total No. of tumours.

centage is 5% at VC 10,000 ppm and 1.7% at 6000 ppm; at this time no tumours were found after administration of the lower concentrations. It is also worthy of note that all malignancies in this series were Zymbal gland carcinomas. After 35 weeks of exposure to 30,000 ppm (Expt BT6) 2 Zymbal gland carcinomas have been observed among 60 rats (58 survivors). In Expt BT5, two groups of 30 mature breeder rats were exposed to VC 10,000 ppm and 6000 ppm for 7 days during pregnancy. No tumours were observed after 69 weeks among the 28 survivors of each group. Angiosarcomas of the subcutaneous tissue occurred in their offspring; one tumour in one of the 54 rats of the 10,000 ppm group at the age of 24 weeks; the other tumor in one of the 36 animals of the 6000 ppm group was found at 22 weeks of age. Fifty and 30 rats, respectively, of the litters are still alive and under observation.

Table 4 indicates that mice respond to VC inhalation of 10,000–50 ppm in a similar manner as rats. While 50 ppm is again ineffective after 35 weeks, 30%, 15%, and 8.4% neoplasms were found after 10,000–6000, 2500–500, and 250 ppm, respectively. It is perhaps not surprising that in mice, pulmonary and mammary tumors were more frequent than other localizations, but the presence of angiosarcomas of the liver also occurred in this genus.

At present, 5 distinct types of tumours induced by VC have been observed, i.e., angiosarcomas, particularly of the liver (in rats and mice), nephroblastomas (in rats), Zymbal gland carcinomas (in rats) and pulmonary adenomas, some of

TABLE 4
EXPERIMENT BT11: RESULTS AFTER 35 WEEKS

Groups and treatment	Animals (Swiss mice) Treated ♂	♀	Total	Survivors ♂	♀	Total	Animals with tumours: Pulmonary tumours No.	Mammary carcinomas[a] No.	Liver angiosarcomas No.	Other type and/or site No.[b]	Total No.[c]
I VC 10,000 ppm	30	30	60	6	18	24	12	4	—	3*	19
II VC 6000 ppm	30	30	60	11	18	29	12	3	1	2*	18
III VC 2500 ppm	30	30	60	11	22	33	7	2	—	—	9
IV VC 500 ppm	30	30	60	13	25	38	7	—	2	—	9
V VC 250 ppm	30	30	60	22	19	41	4	1	—	—	5
VI VC 50 ppm	30	30	60	13	24	37	--	—	—	—	—
VII No treatment	80	70	150	61	61	122		--	—	—	—
Total	269	259	519	137	187	324	42	10	3	5	60

[a] In females.
[b] One skin squamocellular carcin. nos; 1 skin invasive acanthoma; 1 subcutaneous hemangioma;
[c] One skin squamocellular carcinoma; 1 thymic lymphosarcoma.
[c] Total No. of tumors.

which undergo malignant transformation and mammary carcinomas (in mice).

Macroscopic and microscopic illustrations of the tumours induced are shown in Figs. 1–25.

The frequent Zymbal gland carcinomas can reach considerable size (Fig. 1*),[1] are sometimes bilobated and show a tendency to hemorrhages. Their structure can show different patterns. The arrangement of the normal Zymbal gland (Fig. 2) appears often in a disorganized form in the tumours. The carcinomas present various different histological patterns, e.g., glandular (Fig. 3), solid (Fig. 4*), squamous, anaplastic, and polymorphous. The frequent pulmonary metastases carry the characteristics of the primary lesion, (Fig. 5). The nephroblastomas (Figs. 6–8) lead to numerous metastases in the liver (Figs. 9*, 10*) but can also involve spleen and lungs. The frequently found angiosarcomas of the liver in rats (Figs. 11*, 12*, 13*, and 14) are characterized by the vascular growth and hemorrhagic appearance which is also present in pulmonary metastases (Fig. 15*). Occasionally, both angiosarcoma of the liver and nephroblastoma occurred in the same animal (Fig. 16*). Angiosarcomas of other sites appear generally as more or less hemorrhagic masses developing in the ab-

[1] Figures marked by asterisks (*) appear on the color plates.

dominal cavity (Fig. 17), in the subcutaneous tissue (Fig. 22*) in the uterus (Fig. 18), or in the lung (Fig. 19). The ossifying angiosarcoma (Figs. 20*, 21*) and the salivary gland carcinoma (Figs. 23 and 24) occurred rarely (see Table 2, group VI). Figure 25 shows a liver angiosarcoma of a mouse.

CONCLUSIONS AND DISCUSSION

From the results presented in the tables, from the pathological and histological observations, the following early conclusions may be drawn.

(1) VC is oncogenic under our experimental conditions. It induces, in rats, carcinomas of the Zymbal glands, nephroblastomas and angiosarcomas in liver and other sites; in mice, liver angiosarcomas, pulmonary adenomas and mammary carcinomas.
(2) A direct relationship exists between the dose and length of treatment, and the neoplastic response.
(3) Zymbal gland carcinomas and nephroblastomas may be bilateral.
(4) Liver angiosarcomas are often multicentric.
(5) Blood vessel ectasias and endothelial hyperplasia, associated or not with cellular atypia, are often observed in liver and in other organs and tissues in treated animals, with or without angiosarcomas. Therefore, the effect of VC on blood vessels and endothelia should be considered systemic.
(6) The onset of 2 ossifying angiosarcomas suggests a new orientation in the pathogenetic interpretation of acroosteolysis in workers exposed to VC.
(7) To the present no acroosteolytic lesions have been observed in our exposed animals.
(8) No tumours have been observed in rats exposed to VA (2500 ppm).

Zymbal gland carcinomas, nephroblastomas and liver angiosarcomas have never been observed to occur spontaneously in our breed of Sprague–Dawley rats.

Although Zymbal glands, in rats, are responsive to a large number of carcinogens, only 3 spontaneously occurring cases of Zymbal gland carcinomas have been recorded in this species (Tannenbaum *et al.*, 1962). To our knowledge, no spontaneous nephroblastomas and liver angiosarcomas of rats have been reported

FIG. 1. Rat with Zymbal gland carcinoma.
FIG. 4. Zymbal gland carcinoma, solid pattern; in rat. H–E × 158.
FIG. 9. Rat with nephroblastoma; metastases in liver.
FIG. 10. Liver metastasis from nephroblastoma; in rat. H–E × 158.
FIG. 11. Rat with liver angiosarcoma.
FIG. 12. Rat with liver angiosarcoma with lung metastasis.
FIG. 13. Liver angiosarcoma; characteristic pattern; in rat. H–E × 158.
FIG. 15. Pulmonary metastasis of liver angiosarcoma; in rat. H–E × 158.
FIG. 16. Liver angiosarcoma + nephroblastoma in rat.
FIG. 20. Laterocervical ossifying angiosarcoma; in rat. H–E × 158.
FIG. 21. Pulmonary metastasis of ossifying angiosarcoma; in rat. H–E × 40.
FIG. 22. Rat with subcutaneous angiosarcoma.

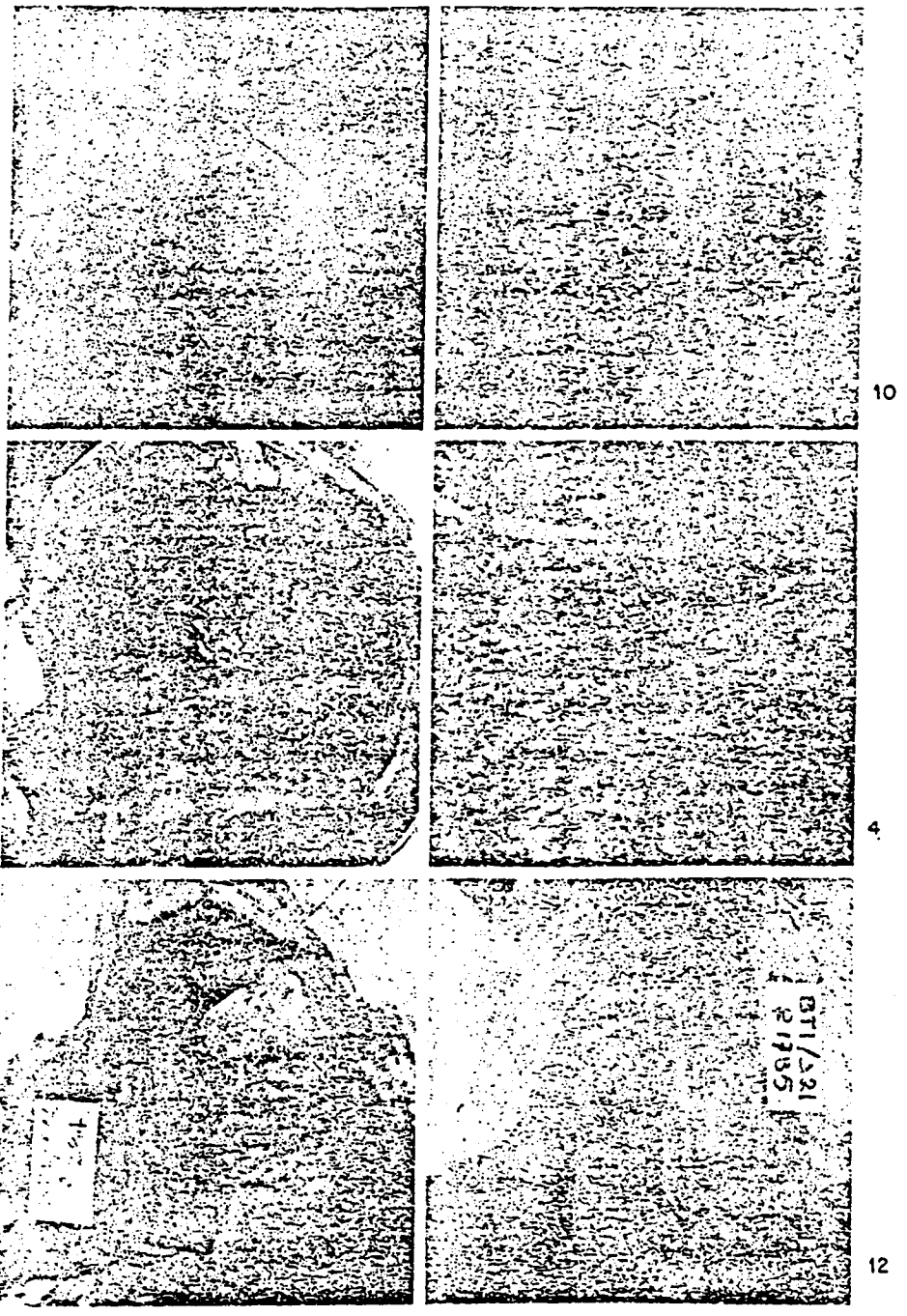

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Fig. 3. 7

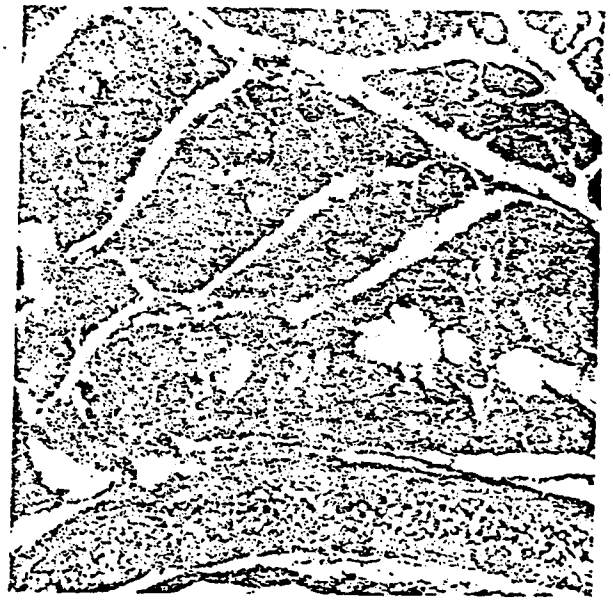

FIG. 2. Normal Zymbal gland of rat: H-E × 46.

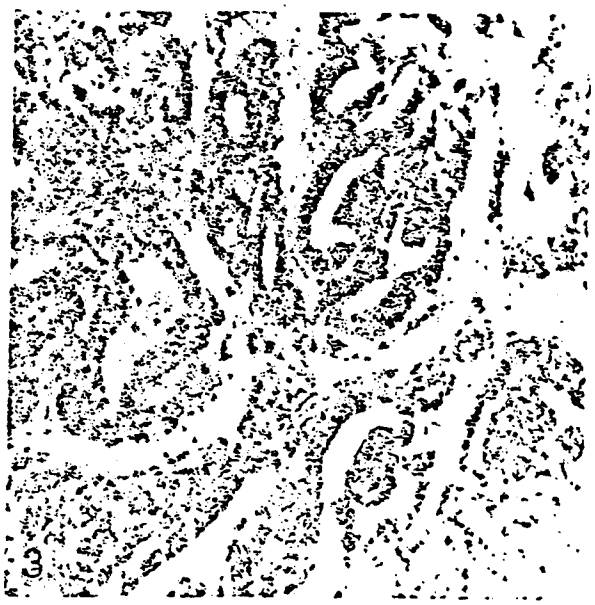

FIG. 3. Zymbal gland carcinoma, glandular pattern in rat: H-E × 155.

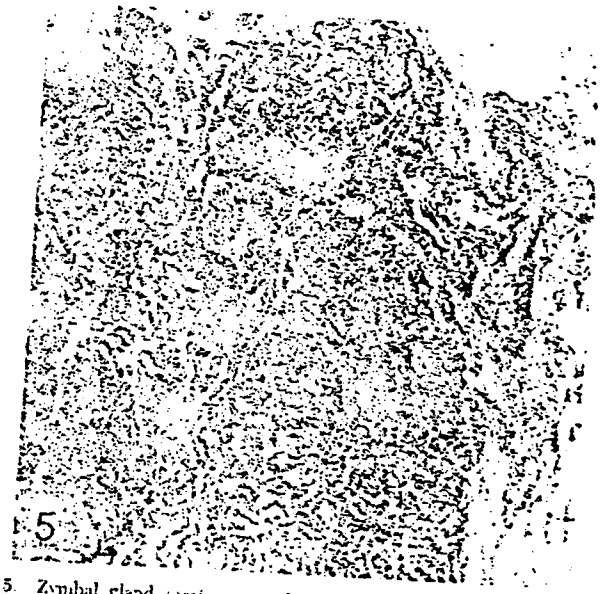

FIG. 5. Zymbal gland carcinoma; pulmonary metastasis in rat. H–E × 46.

FIG. 6. Nephroblastoma in rat.

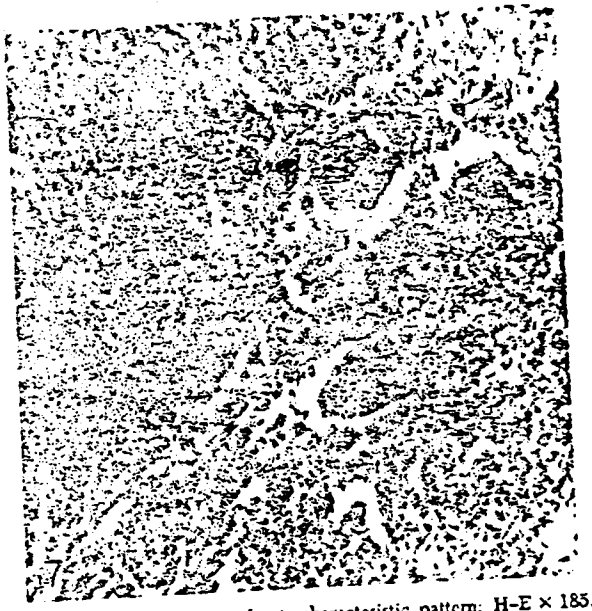

FIG. 7. Nephroblastoma of rat; characteristic pattern; H–E × 185.

FIG. 8. Nephroblastoma of a rat: nephrogenic blastema differentiating in a glomerulus H–E × 470.

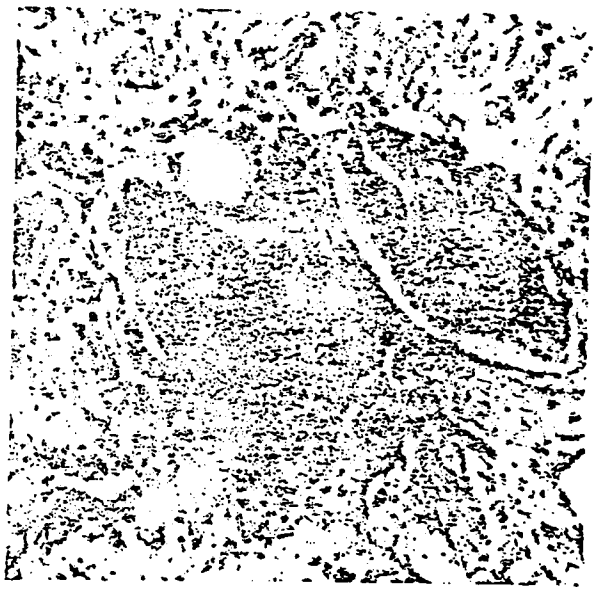

FIG. 14. Liver angiosarcoma; characteristic pattern in a rat. H–E × 185.

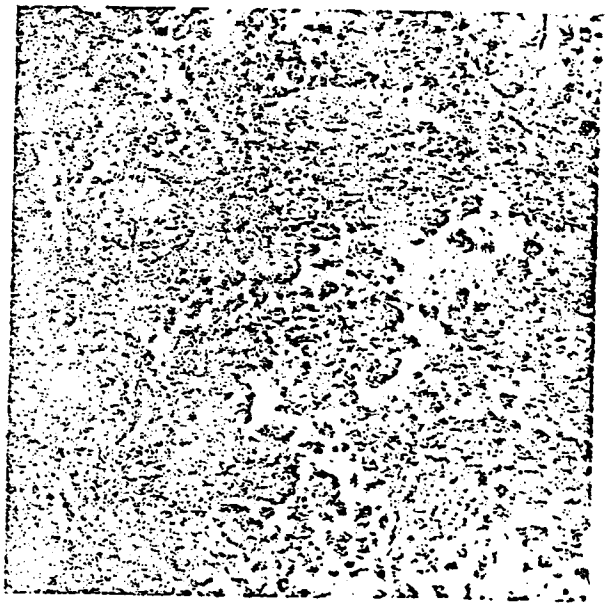

FIG. 17. Intra-abdominal angiosarcoma near the spleen, in rat. H–E × 185.

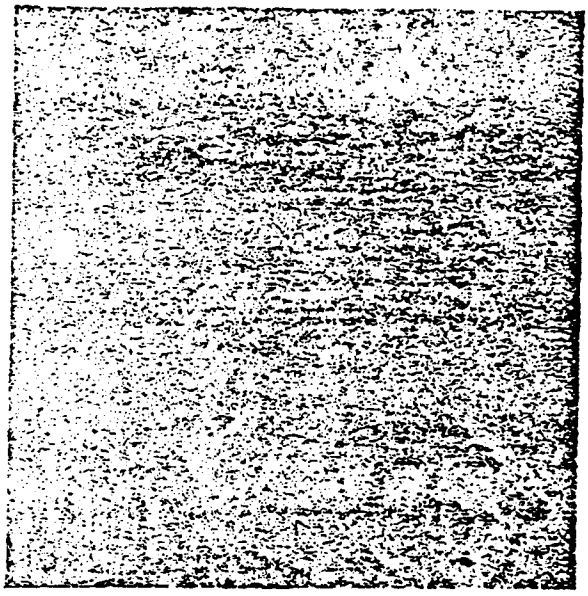

FIG. 18. Uterus angiosarcoma; in rat. H–E × 470.

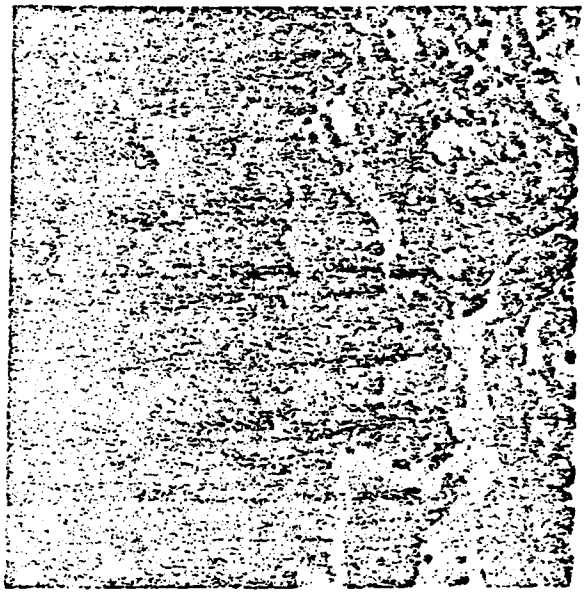

FIG. 19. Angiosarcoma of lung, in rat. H–E × 185.

FIG. 23. Rat with salivary gland adenocarcinoma.

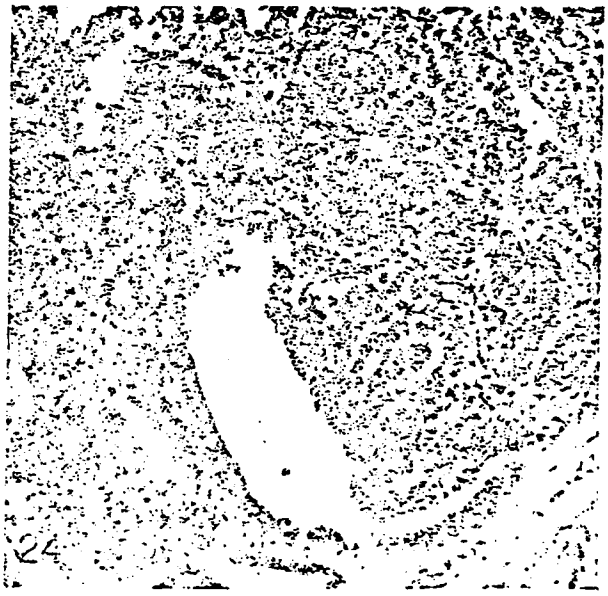

FIG. 24. Salivary gland adenocarcinoma; in rat. H-E × 185.

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FIG. 25. Liver angiosarcoma in a mouse. H–E × 185.

in literature and as far as we know, kidney nephroblastomas have never been reported as being experimentally induced.

Hepatic angiosarcomas have been experimentally induced in rats (Reuber and Lee, 1968; Hadjiolov, 1972), in mice (Roe and Salaman, 1954; Cardesa *et al.*, 1973) and in hamsters (Toth, 1972). In mice, extrahepatic angiosarcomas have been induced by *o*-aminoazotoluene (Andervont *et al.*, 1942; Andervont, 1950) and by *p*-dimethylaminobenzene-1-azo-2-naphthalene (Mulay and Saxén, 1953). In the rabbit, angiosarcomas have been produced by thorotrast (Zeitlhofer and Speiser, 1954).

In man, angiosarcomas are quite rare. One hundred-seventeen documented cases were collected to 1958 (Landells, 1958), of which only 21 arose in the liver.

Induction of liver angiosarcomas have been reported in humans. In one case, a liver angiosarcoma followed the insertion of a radium needle (Ross, 1932). Cases of liver angiosarcomas have been reported following administration of thorotrast (MacMahon *et al.*, 1947; Lüdin, 1953; Baserga *et al.*, 1960; Da Silva Horta *et al.*, 1965) and exposure to arsenic (Roth, 1957).

The results of our investigations have been periodically transmitted to the members of the European Cooperative Group. When, approximately a year and a half ago, it became definitely clear that VC was able to induce angiosarcomas, nephroblastomas and other malignancies, our results were also communicated to major American manufacturers of VC and PVC, to orient clinical observations

and epidemiological investigations, and to guide industrial hygiene measures needed for prevention of diseases which might be associated with VC exposure.

SUMMARY

This project of investigations is concerned with determining the oncogenic potentialities of vinyl chloride (VC). The effect of this compound is studied in relation to various experimental factors, such as route of administration, dose level, length of treatment, and species, strain and age of the animals.

The preliminary results are presented. When given by inhalation VC induces, in rats, Zymbal gland carcinomas, nephroblastomas and angiosarcomas of the liver and of other anatomical sites, and in mice, pulmonary adenomas, mammary carcinomas, and liver angiosarcomas.

A direct relationship between dose and length of treatment, and neoplastic response has been found.

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